Conference Organizers

Conference Chair Inga Ciprovica, Latvia University of Agriculture, LV

Editorial Board
Dr.sc.ing. Tatjana Rakejejeva, Latvia University of Agriculture, LV
Dr. Joerg Hampshire, Fulda University of Applied Sciences, DE
Dr. Ase Solvej Hansen, Copenhagen University, DK
Dr.sc.ing. Daina Karklina, Latvia University of Agriculture, LV
Dr. Raija Lantto, VTT Technical Research Centre of Finland, FIN
Dr.med.sc. Aleksandr Shleikin, Saint-Peterburg National Research University of Information Technologies, Mechanics and Optics, RUS
Dr.sc.ing. Evita Straumite, Latvia University of Agriculture, LV
Dr. Thierry Talou, National Polytechnic Institute of Toulouse, FR
Dr. Rimantas Venskutonis, Kaunas University of Technology, LT
Dr.biol. Raivo Vokk, Tallin Technological Institute, EE
Dr. Erkan Yalcin, Abant Izzet Baysal University, TR

Organising Committee
Chair Ruta Galoburda, Latvia University of Agriculture, LV
Zanda Kruma, Latvia University of Agriculture, LV
Dace Klava, Latvia University of Agriculture, LV
Irisa Murniece, Latvia University of Agriculture, LV

Editor-in-chief and responsible compiler of Conference Proceedings – leader researcher Evita Straumite

Printed and bound in Riga by „Drukātava”

© LLU, Faculty of Food Technology, 2014
Dear participants,

The 9th Baltic Conference on Food Science and Technology „Food for consumer well-being” (FoodBalt 2014) will take place from 8th to 9th May, 2014 in the Latvia University of Agriculture (LLU). The conference is organized by the Faculty of Food Technology. The conference aims are to bring together master and doctoral students from Baltic countries, as well as students, established researchers from other countries, promoting the dissemination of new knowledge and allowing young scientists and students to meet professionals, contributing to the development of food science and technology area.

The conference plans to attract more than 120 delegates from 10 countries. The conference programme contains 4 Key lectures and 35 oral presentations over 7 sessions. Additionally, a total of 94 posters will be presented. The conference Organising Committee had received around 70 full paper submissions from 7 countries. A peer review process was enforced, with the help of experts who were members of the Conference Scientific Committee and researchers from Latvia University of Agriculture, all of them internationally recognized in one of the main conference topic areas. 69 papers were selected for publishing and published as reviews (4), original papers (57) and short communications (8).

On behalf of Organizing Committee I am honoured to welcome you at FoodBalt 2014 conference and to wish new experience, contacts and further cooperation activities in common projects and programmes. It would help you to achieve the excellence and recognition in food science area in Baltic region and in Europe, too.

Dr.sc.ing. Inga Ciprovica
The chair of the 9th Baltic conference on Food Science and Technology
CONTENTS

Reviews
Sebahattin Serhat Turgut, Erkan Karacabey, Erdoğan Küçükoğlu POTENTIAL OF IMAGE ANALYSIS BASED SYSTEMS IN FOOD QUALITY ASSESSMENTS AND CLASSIFICATIONS ................................................................. 8
Rudite Sausserde, Kaspars Kampuss COMPOSITION OF CAROTENOIDS IN CALENDULA (CALENDULA OFFICINALIS L.) FLOWERS....................................................... 13
Friedrich-Karl Lücke, Sonja Schreiber HIGH-OXYGEN MODIFIED ATMOSPHERE PACKAGING OF ORGANIC MEATS?.............................................................. 19
Özgül Özdestan, Tuğçe Erol, Burcu Acar PHYTOSTEROLS IN RICE BRAN AND USAGE OF RICE BRAN IN FOOD INDUSTRY ................................................................. 24

Original Papers
Daina Ikauniece, Aleksandrs Jemeljanovs, Vita Sterna, Vita Strauzdina EVALUATION OF NUTRITION VALUE OF ROMAN SNAIL’S (HELIX POMATIA) MEAT OBTAINED IN LATVIA ................................................................. 28
Vita Straudzina, Aleksandrs Jemeljanovs, Vita Sterna, Daina Ikauniece NUTRITIONAL CHARACTERISTICS OF WILD BOAR MEAT HUNTED IN LATVIA .................................................. 32
Ilze Gramatīna, Laima Silina, Liga Skudra, Tatjana Rakcejeva DRIED VENISON PHYSICAL AND MICROBIOLOGICAL PARAMETERS CHANGES DURING STORAGE ................................................................................................................ 37
Lilija Degola THE DIFFERENT PROTEIN SOURCES FEEDING IMPACT ON THE QUALITY OF PORK............................................................................................................ 42
Modestas Ružauskas, Lina Vaškevičiūtė, Rita Šiugždinienė, Marius Virgailis, Rita Plančiūnienė, Lina Anskienė, Raimundas Mockeliūnas, Alvydas Pavilonis ANTIMICROBIAL SUSCEPTIBILITY OF OXACILLIN-RESISTANT STAPHYLOCOCCUS SPP. ISOLATED FROM POULTRY PRODUCTS ............................................................. 47
Sónia C. Andrade, Raquel P. F. Guiné, Luísa C. P. Roseiro DETERMINATION OF THE CONTENT OF COENZYMES Q9 AND Q10 IN PORK MEAT FROM DIFFERENT BREEDS ................................................................................................................ 51
Indulis Silins THE EFFECTS OF pH, aw, AND LACTIC ACID BACTERIA ON LISTERIA MONOCYTOGENES IN FERMENTED SAUSAGES ........................................................................... 55
Aija Melngaile, Elina Ciekure, Olga Valcina MICROBIOLOGICAL QUALITY OF MEAT PREPARATIONS AND MEAT PRODUCTS ................................................................................................................................. 61
Anda Linina, Antons Ruza, Daiga Kunkulberga, Tatjana Rakcejeva THE INFLUENCE OF ENVIRONMENTAL CONDITIONS ON WINTER WHEAT WHOLEMEAL PROTEIN CONTENT AND RHEOLOGICAL PROPERTIES ........................................................................................................... 66
Linda Brunava, Ina Alsina, Sanita Zute, Vita Sterna, Zaiga Vicupe SOME CHEMICAL, YIELD AND QUALITY PROPERTIES OF DOMESTIC OAT CULTIVARS ........................................................................................................... 72
Vita Sterna, Sanita Zute, Linda Brunava, Zaiga Vicupe LIPID COMPOSITION OF OAT GRAIN GROWN IN LATVIA ................................................................................................................................. 77
Sindija Zeberga, Maija Strele, Anna Vojevodova, Inese Mierina, Mara Jure OAT HULLS AND SEA BUCKTHORN POMACE – A POTENTIAL SOURCE OF ANTIOXIDANTS FOR HEMPSEED OIL ........................................................................... 81
Kristina Antonenko, Viesturs Kreichbergs, Anda Linina, Daiga Kunkulbeği INFLUENCE OF DIFFERENT SELENIUM CONCENTRATIONS ON THE PROTEIN AND STARCH CONTENT IN RYE MALT ........................................................................ 86
Liene Jaundzeikare, Ilze Beitane COMPARISON OF DIETARY FIBRE CONTENT IN DIFFERENT FIBRE SOURCES......................................................... 90

Polina Smolikhina, Evgeniia Muratova DETERMINATION OF THE OPTIMAL RATIO OF RECIPE INGREDIENTS IN THE PROCESS OF DESIGNING CONFECTIONERY PRODUCTS.......................................................................................................................... 94

Lija Dukalska, EvaUngure, Sandra Muizniec-Brasava INTERACTION OF SUGAR CONFECTIONERY SHERBET QUALITY PARAMETERS DURING STORAGE TIME IN DIFFERENT PACKAGING......................................................................................................................... 99

Polina Smolikhina, Evgeniia Muratova TECHNOLOGICAL ASPECTS OF THE RESULTS ON RHEOLOGICAL STUDIES OF CANDY MASS...................................................... 105

Iveta Pugajeva, Laura Zumbure, Aija Melngale, Vadiems Bartkevics DETERMINATION OF ACRYLAMIDE LEVELS IN SELECTED FOODS IN LATVIA AND ASSESSMENT OF THE POPULATION INTAKE.............................................................................................................. 111

Daiga Kunkulberga, Ilga Gedrovica, Vija Ozolina, Inga Ciprovica, Vita Stermara ACRYLAMIDE REDUCTION OPTIONS IN RYE BREAD.......................................................................................................................... 117

Santa Senhofa, Evita Straumite, Dace Klava QUALITY CHANGES OF CEREAL MUESLI WITH SEEDS DURING STORAGE............................................................................................... 123

Vitalijs Radenkovs, Dace Klava, Inta Krasnova, Karina Juhnevica-Radenkova APPLICATION OF ENZYMIC TREATMENT TO IMPROVE THE CONCENTRATION OF BIOACTIVE COMPOUNDS AND ANTIOXIDANT POTENTIAL OF WHEAT AND RYE BRAN..................................................................................................................... 127

Solvita Kalnina, Tatjana Rakcejeva, Ilze Gramatina, Daiga Kunkulberga INVESTIGATION OF TOTAL DIETARY FIBER, B1, AND B2 VITAMIN CONTENT OF FLOUR BLEND FOR PASTA PRODUCTION........................................................................ 133

Asnate Kirse, Daina Karklina NUTRITIONAL EVALUATION OF PULSE SPREADS IN COMPARISON TO NUTRIENT RECOMMENDATIONS.................................................................................. 138

Liene Strauta, Sandra Muizniec-Brasava, Ina Alsina, Tatjana Rakcejeva EXTRUDED BEAN PRODUCT QUALITY EVALUATION .......................................................................................... 144

Ingrida Augspole, Tatjana Rakcejeva, Zanda Kruma, Fredijs Dimins SHREDDED CARROTS QUALITY PROVIDING BY TREATMENT WITH HYDROGEN PEROXIDE....................................................................................................................... 150

Ona Bundiené, Vytautas Zalatorius, Danguolė Kavaliauskaitė, Roma Starkutė EFFICIENCY OF THE ADDITIONAL FERTILIZATION WITH NITROGEN FERTILIZERS GROWING CARROT OF EXCEPTIONAL QUALITY AND ITS INFLUENCE ON PRODUCTION STORAGE........................................................................... 155

Karina Juhnevica-Radenkova, Liga Skudra, Mara Skrivele, Vitalijs Radenkovs, Dalija Seglina IMPACT OF THE DEGREE OF MATURITY ON APPLE QUALITY DURING THE SHELF LIFE.............................................................................................................. 161

Karina Ruse, Tatjana Rakcejeva PHYSICAL AND CHEMICAL PARAMETERS OF LATVIAN FRESH CRANBERRIES.......................................................................................................................... 167

Ruta Galoburda, Sigita Boca, Imants Skrupsks, Dalija Seglina PHYSICAL AND CHEMICAL PARAMETERS OF STRAWBERRY PUREE.................................................................................. 172

Rita Riekstina-Dolge, Zanda Kruma, Ingmars Cinkmanis, Evita Straumite, Martins Sabovics, Lolita Tomsoniene INFLUENCE OF OENOCCOCCUS OENI AND OAK CHIPS ON THE CHEMICAL COMPOSITION AND SENSORY PROPERTIES OF CIDER.................................................................................................................... 178

Ingmars Cinkmanis, Sanita Vucane, Ilze Cakste BERRY AND FRUIT JUICES AS POTENTIAL UNTRADITIONAL ACIDITY REGULATORS IN MASHING.................................................................. 184

Ivo Lidums, Daina Karklina, Asnate Kirse QUALITY CHANGES OF NATURALLY FERMENTED KVASS DURING PRODUCTION STAGES........................................................................ 188
Lolita Tomsone, Zanda Kruma INFLUENCE OF FREEZING AND DRYING ON THE PHENOL CONTENT AND ANTIOXIDANT ACTIVITY OF HORSERADISH AND LOVAGE................................................................. 192
Liga Priecina, Daina Karklina DETERMINATION OF MAJOR SUGARS IN FRESH AND DRIED SPICES AND VEGETABLES USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY........................................................................... 198
Ayca Aydogdu, Gulum Sumnu, Serpil Sahin PORE SIZE DISTRIBUTION OF EGGPLANTS DRIED BY DIFFERENT DRYING METHODS............................. 202
Irisa Murniece, Lolita Tomsone, Ilze Skrabule, Aija Vaivode CAROTENOIDS AND TOTAL PHENOLIC CONTENT IN POTATOES WITH DIFFERENT FLESH COLOUR.... 206
Solvita Kampuse, Aleksandrs Jefimovs, Tatjana Rakcejeva THE INFLUENCE OF PRE-TREATMENT METHOD ON THE FAT CONTENT DECREASE IN FRENCH FRIES................................................................................................. 212
Aija Ruzaike, Sandra Muizniece-Brasava, Lija Dukalska PACKAGING MATERIAL AND STORAGE-INDUCED QUALITY CHANGES IN FLEXIBLE RETORT POUCH POTATOES’ PRODUCE................................................................. 217
Lina Nuobariene, Nils Arneborg, Åse Solvej Hansen PHYTASE ACTIVE YEASTS ISOLATED FROM BAKERY SOURDOUGHS.............................................................. 223
Martins Sabovics, Evita Straumite, Ruta Galoburda THE INFLUENCE OF BAKING TEMPERATURE ON THE QUALITY OF TRITICALE BREAD................................. 228
Ilze Beitane, Gita Krumina-Zentmere, Irisa Murniece SENSORY, COLOUR AND STRUCTURAL PROPERTIES OF PANCAKES PREPARED WITH PEA AND BUCKWHEAT FLOURS................................................................................. 234
Ilkem Demirkesen Mert, Osvaldo H. Campanella, Gulum Sumnu, Serpil Sahin GLUTEN-FREE SOURDOUGH BREAD PREPARED WITH CHESTNUT AND RICE FLOUR.............................................................................................. 239
Banan Koç, Figen Kaymak-Ertekin THE EFFECT OF SPRAY DRYING PROCESSING CONDITIONS ON PHYSICAL PROPERTIES OF SPRAY DRIED MALTODEXTRIN................. 243
Mara Kuka, Ilze Cakste, Ruta Galoburda, Martins Sabovics CHEMICAL COMPOSITION OF LATVIAN WILD EDIBLE MUSHROOM CANTHARELLUS CIBARIUS..................................................................................................... 248
Aleksandrs Kovalcuks, Mara Duma SOLVENT EXTRACTION OF EGG OIL FROM LIQUID EGG YOLK........................................................................................................ 253
Ashlı Zungur, Mehmet Koç, Buket Yaşçın, Figen Kaymak-Ertekin, Semih Ötleş STORAGE STABILITY OF MICROENCAPSULATED EXTRA VIRGIN OLIVE OIL POWDER...................................................................................... 257
Mara Duma, Ina Alsina, Solvita Zeipina, Liga Lepse, Laila Dubova LEAF VEGETABLES AS SOURCE OF PHYTOCHEMICALS.................................................. 262
Jana Feldmane, Inga Ciprovica, Pavels Semjonovs, Raimonds Linde THE INFLUENCE OF FERMENTATION TEMPERATURE ON THE DEVELOPMENT OF EXOPOLYSACCHARIDES IN YOGHURT PRODUCTION.................................................. 266
Raquel P. F. Guiné, Cáitia F. F. Almeida, Paula M. R. Correia EVALUATION OF PRESERVATION CONDITIONS ON NUTS PROPERTIES...................................... 271
Grazina Juodeikiene, Loreta Basinskiene, Daiva Vidmantiene, Dovile Klupsaite, Elena Bartkiene THE USE OF FACE READING TECHNOLOGY TO PREDICT CONSUMER ACCEPTANCE OF CONFECTIONERY PRODUCTS............................................. 276
Paula M. R. Correia, Susete Gonçalves, Helena Gil, Jorge Coelho THE WETTABILTY AND SURFACE FREE ENERGY OF ACORN STARCH GELS ISOLATED BY ALKALINE AND ENZYMATIC METHODS...................................................... 280
Fredijs Dimins, Velga Mikelsone, Peteris Kuka, Arturs Niklavs Jefremovs EFFECT OF DIFFERENT TYPES OF HEAT TREATMENT ON INVERTASE ACTIVITY IN HONEY

Perihan Kendirci, Tomris Altug Onogur COMPARISON OF THREE DIFFERENT TECHNIQUES FOR EXTRACTION OF VOLATILES FROM PISTACHIO NUTS

Ausra Simoneliene, Ernesta Treciokiene, Giedre Lukosiunaite, Gediminas Vysniauskas, Ernesta Kasparaviute RHEOLOGY, TECHNOLOGICAL AND SENSORY CHARACTERISTICS OF FORTIFIED DRINK PRODUCTS WITH FIBERS

Ivan Shatalov, Alexandrina Shatalova, Aleksandr Shleikin DEVELOPING OF EDIBLE PACKAGING MATERIAL BASED ON PROTEIN FILM

Tatjana Tokareva, Aija Eglite NON-PRICE FACTORS THAT INFLUENCE CONSUMERS' WASTED FOOD AMOUNTS

Emine Yilmaz, Yasemin Oraman, Gokhan Unakitan, Burcin Basaran CURRENT EATING PATTERNS AND LIFESTYLE ON THE HEALTH OF NAMIK KEMAL UNIVERSITY STUDENTS

Short Communications

Ruta Budreckiene, Agne Struzeckiene OPTIMIZATION OF MANUFACTURING TECHNOLOGY OF SOFT CHEESE

Dmitry Pyanov, Artem Delmukhametov, Evgeny Khrustalev PIKE-PERCH FARMING IN RECIRCULATING AQUACULTURE SYSTEMS (RAS) IN THE KALININGRAD REGION

Ismail Yilmaz, Hafize Garipoğlu DETERMINATION OF AFLATOXIN CONTAMINATION IN SOME DRIED NUTS AND SPICES BY ELISA

Ismail Yilmaz, Mehmet Aluc DETERMINATION OF AFLATOXIN LEVELS IN CASHEWS ON TURKISH MARKETS

Ance Dandena, Liga Zuka, Maria Kostova NATURAL ANTI-OXIDANTS IN BLACK CHOKEBERRY MARC EXTRACTS DEPENDING ON THE EXTRACTION METHOD

Maria Joao Barroca, Susana A. Séro, Raquel P. F. Guiné, Paula M. R. Correia INFLUENCE OF PRE DRYING TREATMENT ON PHYSICAL PROPERTIES OF CARROTS

Ömer Güvendi, Erkan Yalçın EGG PASTA (ERİŞTE) PRODUCED FROM WHOLE GRAIN OAT FLOUR

Alvija Salasevičienė, Lina Vaiciulyte-Funk, Irina Koscelkovskienė IMPACT OF LOW TEMPERATURE, PROLONGED TIME TREATMENT AND VACUUM DEPTH ON THE PORCINE MUSCLE QUALITY AND SAFETY

Meinolf G. Lindhauer STANDARD METHODS AND CRITERIA TO PREDICT BREAD CEREAL QUALITY – DO THEY STILL MEET THE DEMANDS OF MODERN RAW MATERIALS AND THEIR PROCESSING?

Yasemin Oraman, Emine Yilmaz, Gokhan Unakitan, Burcin Basaran ANALYTIC STUDY OF UNIVERSITY STUDENTS’ NUTRITIONAL HABITS AND ATTITUDES AS A PART OF SOCIETY IN TURKEY

Authors List
**REVIEW**

**POTENTIAL OF IMAGE ANALYSIS BASED SYSTEMS IN FOOD QUALITY ASSESSMENTS AND CLASSIFICATIONS**

Sebahattin Serhat Turgut, Erkan Karacakay, Erdoğan Küçüköner

Food Engineering Department, Engineering Faculty, Suleyman Demirel University, Isparta, Turkey,
e-mail: erdogankucukoner@sdu.edu.tr

**Abstract**

Increasing life standards, developing technology, growing importance of food quality and safety lead food industry to search new analysis methods. In addition, high consumer expectation and increasing population forces manufacturers to find fast and accurate techniques. The present study was aimed to prepare a summary about computer aided image processing systems covering working principles and applications for different analysis of food products. Image processing systems have recently been considered in this extent and results have revealed that computer aided these techniques can provide all these needs in a non-destructive way for samples. These techniques can be adapted to a wide range of food and agriculture products like meat, bakery products, dairy products, vegetables but primarily fruits. Image processing can be utilized for different purposes related to these product groups. Size and shape based classification, defects detection, microbial safety, quality grading and variety determination are mainly investigated topics. Literature survey indicates favourable reported results. Generally small scale investigations have been presented, but some of them could find a place for industrial application with high success. Computer aided image processing has been taken increasing interests of researcher for its potential in the applications for food technologies.

In the present study it was aimed to prepare a summary about computer aided image processing systems covering working principles and applications for different analysis of food products. As a conclusion it could be said that computer aided image processing systems reveal high potential in food industry.

**Keywords:** image processing, fast analysis method, food quality, classification.

**Introduction**

The developments in the technology, media and communication accompany the increasing awareness of consumers. Therefore, there are more expectations than ever. The situation forces the manufacturers to produce and present higher quality food and agricultural products to market. Moreover, these products have to satisfy sophisticated consumer desires. Computer imaging system is one of the methods serving the assurance for high quality food products.

Product quality is evaluated by a wide range of parameters including external, internal parameters. However, in some cases, sensory and safety scores gain higher importance than above ones. External quality parameters, such as surface colour, texture, presence of bruises and defects, are generally monitored and sorted manually by workers, whereas the internal quality parameters including firmness, pH value, soluble solid contents, titratable acidity are evaluated using common techniques. Sensory (e.g. sweetness, flavour) and food safety (e.g., pathogenic bacteria and faecal contamination, pesticide residues and other hazardous residues) characteristics influence general palatability of the products. However, the old fashion techniques are time consuming, destructive and unable to represent the whole batch (Zhang et al., 2012). Unlike to traditional ones, computer imaging systems do not cause any damage on/in the product and they are rapid analysis techniques as well as being feasible for in-line process (Kim et al., 2007; Park et al., 2011).

Being another advantage, these systems in this extent can be easily implemented for any analysis of an individual object and/or a batch of food and agriculture products, like intact fruits, even as they are on the

yards (Stajnko et al., 2004). Against the traditional techniques, intensity of the analysed feature in a bulk is possible to figure out by imaging systems (ElMasry et al., 2012, Feng et al., 2013). Moreover, these systems also provide opportunity to perform rapid, hygienic, automated and objective inspections.

This paper represents an overview on camera imaging systems in the food industry. Basic knowledge is provided about steps and principles, some examples of applications are submitted as well.

**Basic Steps of Image Processing and Analysis**

Computer vision is a system designed with a few equipment and algorithm which are used to obtain information about objects of interest. The information could be utilized in order to shape classification, quality sorting, identification of internal and external features, etc.

Recognition processes of the specialities of the objects occur with a series of steps. Image acquisition, preprocessing, segmentation, representation and description, recognition and interpretation are basically the steps of the computer vision processes and every step must be implemented carefully, or else the results might be unsatisfactory (Gunasekaran, 1996).

Image acquisition is the stage for turning electronic signals from sensing device into numeric form. Ultrasound, X-ray and near infrared spectroscopy, displacement devices and document scanners, solid state charged coupled device (CCD) cameras are some of the sensors used to create images (Brosnan, Sun, 2004). Thermal imaging cameras (Gowen et al., 2010) and terahertz cameras are also used in image analyses systems (Lee, Lee, 2014). In order to get a high quality image which is a vital factor for sequent
steps, illumination and lighting arrangement, high quality optics and electronic circuitry must be prepared properly (Gunasekaran, 1996). Light sources vary according to purpose of using such as incandescent, fluorescent, lasers, X-ray tubes and infrared lamps (Brosnan, Sun, 2004).

Pre-processing stage includes one or more operations of noise reduction, geometrical correction, grey-level correction and correction of defocusing and aims to improve image quality (Shirai, 1987). Image segmentation is one of the most important stages because accuracy of following step deeply related with this step. It is intended to separate the image into parts which have a strong relation with the object (Brosnan, Sun, 2004). After segmentation, the image generally represents a boundary or a region. First types of images are suitable for size and shape analyses while the latter one is used for determination texture and defects. The image representation should be chosen according to planned application (Gunasekaran, 1996). Recognition and interpretation are generally performed using statistical classifiers or multilayer neural networks to provide information that is useful for process or machine control needed for quality sorting and grading (Brosnan, Sun, 2004). Fuzzy logic, decision tree and genetic algorithm are also used as learning techniques the same purposes (Du, Sun, 2006).

Applications in Food Quality Assessments and Classifications

Computer imaging systems can be used for sorting products, detecting defects, identifying internal and external characteristics of foods and inspecting food production equipment, etc. There are a wide range of applications for computer aided analysis systems and their popularities continuously increase. Because of increasing popularity and applicability, some food quality and food process applications were presented in this review.

Fruits and Vegetables

The external appearance of fresh fruits and vegetables is one of the factors affecting the consumer perception. Because the first examination of consumer is visual for quality features such as freshness, taste, decayed, maturity, it has vital importance how to present food and agriculture products to the market. Computer aided vision systems are considered as new tools which are implemented to meet the quality requirements depending on customers’ demands. By this way it is possible to achieve shape classification, defect detection, quality grading and variety classification (Brosnan, Sun, 2004).

Yang et al. (2012) investigated the detection possibilities of faecal contaminations on the surface of Golden Delicious apples using hyperspectral line scan fluorescence imaging and developed a simple multispectral algorithm. A pair of violet red line lights equipped in order to excite the faecal contamination spots on the apples created in different dilutions. The algorithm utilized the four fluorescence densities at four wavebands (680, 684, 720 and 780). It was noted that more than 99% of the faecal spots could detected from the uncontaminated apple areas. It is feasible to use this system on high-speed apple processing line with the purpose of preventing food originated illness for food assurance and reduction of risks. Kim et al. (2007) built up a rapid online scanning system which works both with hyperspectral Vis/NIR reflectance and fluorescence in the Vis with UV-A excitation. The system was combined with a commercial apple sorting machine at a processing line speed over three apples per second. Great performance was acquired with a faecal detection rate of 100% (with no false positive) and 99.5% (with 2% false positive rate only) using fluorescence imaging and near-infrared reflectance, respectively. Another hyperspectral imaging system was developed by ElMasry et al. (2008) for detection of bruises on McIntosh apples in a recent study. The spectral region of the system was between 400 and 1000 nm however three wavelengths in the near infrared region (750, 820, 960 nm) gave promising results. It was noted that the bruised apples were successfully detected from the sound apples. Being different from previous studies, the system could recognize early bruises created just 1 hour before and could also recognize even the apples have different background colours (red, green or reddish).

Wang et al. (2011) examined the possibilities of detecting external insect infestation in jujube fruit. They utilized a hyperspectral reflectance imaging approach in the spectral region of 400–720 nm although three wavelengths (500, 650, 690 nm) gave the maximum discrimination. The results revealed that all of the intact cheeks and calyx-end regions classified correctly. More than 98.0% of the undamaged jujube fruits and 94.0% of the insect-infested jujube fruits were correctly identified and the overall classification success of the system was about 97.0%.

Taghizadeh et al. (2010) focused the evaluation of shelf-life of fresh white mushrooms (Agaricus bisporus) stored in different packaging materials. At the end of the study they managed to determine superiority of packaging materials compared to each other using hyperspectral imaging system. They were able to reduce the colour inspection time less than 1 minute without touching samples and also determined the distribution of quality in a batch which is not possible with classical colourimeters.

Due to hardness of selecting slight rottenness on the mandarins, workers carry out the issue manually under ultraviolet illumination. Often the rottenness is happening by infection of Penicillium sp. fungi. Unless the organisms are eliminated, they can spread to a large number of fruits and can cause great economical loss. Gómez-Sanchis et al. (2008) investigated the possibility of detecting fruits that were rotten due to Penicillium digitatum. The study aimed both preventing workers against the harmful effects of UV lights and detecting troubled mandarins in a faster and more efficient way.
more accurate way. Therefore they used a machine vision system, without any UV radiation illumination. The rate of success for sorting rotten mandarins from sounds was above 91% and this result indicated that the hyperspectral computer vision system was able to detecting damage caused by 
*Penicillium digitatum* in mandarins.

It is also possible to establish some internal quality attributes of fruits and vegetables without damaging via using computer vision system. A study conducted by ElMasry et al. (2007) indicated that moisture content, the total soluble solids and pH value of strawberry fruits could be determined by hyperspectral imaging in and near-infrared (400–1000 nm) regions with high correlation coefficients. Moreover, they conducted a texture analysis on the images based on grey-level co-occurrence matrix (GLCM) in order to determine the ripeness level of the strawberries. High classification accuracy of 89.61% was accomplished using the GLCM parameters. More recently, soluble solid content and titratable acidity, which are two of the internal quality properties indicating tomato flavour, determined by Flores et al. (2009) using near-infrared reflectance spectroscopy. The technique allows the examiner to analyse each tomato sample individually without destroying both in on-set and on-line systems and could accurately estimate tomato quality parameters. Fernandes et al. (2011) examined the determination of anthocyanin concentration of whole grape using hyperspectral imaging and neural network. Hyperspectral data was obtained from whole Cabernet Sauvignon grapes in the reflectance mode between the wavelengths from 400 to 1000 nm. At the end the study, they found a squared correlation coefficient of 0.65 between the estimated and traditionally analysed results.

One of the other techniques used for computer imaging is using thermal cameras. The thermal imaging method developed by Stajnko et al. (2004), in order to predict the number of apples in the orchard and measuring their diameter, brought out successful results. Coefficients of determination ($R^2$) were calculated from 0.83 to 0.88 for numbers of apples and were 0.68 to 0.70 for diameter of apples between manually measured and estimated numbers. It is noted that the rise in both of the correlation coefficient number were seen during the ripening period. Gan-Mor et al. (2011) utilized thermal imaging systems with the purpose of optimizing the high-temperature surface heat treatment which was employed against *Sclerotinia sclerotiorum* (Lib.) de Bary fungi in carrot packaging process. Excessive heating causes damages and discolouration whereas insufficient heating results in incomplete disinfection. The aim of the system was monitoring the process, regulating heat steam level, and gaining uniformity over carrots surface segments using a short, 3 s steam treatment. Reduction around 60–80% in post-storage phytoxotic colour change was achieved and sensitivity to post-storage soft rots by *Sclerotinia sclerotiorum* was lowered significantly.

**Meat**

Computer imaging systems are also applicable in the meat industry. These systems can be used for defining some quality features of fresh beef meats like surface colour, pH and tenderness, contaminants. Park et al. (2011) successfully managed to detect small amount (about 10 mg) of faecal and ingesta contaminants on poultry carcasses by using real-time hyperspectral imaging system. This additional process was found not to affect real time process duration which was about 140 carcasses per min. ElMasry et al. (2012) determined some quality characteristics (pH, colour and tenderness) of fresh beef with traditional methods and modelled with their matching spectral values, gained by a hyperspectral imaging system in the near-infrared spectral region (900–1700 nm), using partial least square regression. However, the authors of this study stated that more samples were required for better examination and more robust modelling, and reported that the presented sampling was just enough to take satisfying results. Good performances were achieved and it was explained the system could be useful, reliable, non-destructive and rapid alternative to traditional analysis methods. Potential applicability under the visible light is one of the other advantages of the technique. Recently great accuracy was found in the study by Feng et al. (2013) where determination coefficients ($R^2$) of *Enterobacteriaceae* bacteria on chicken fillets were 0.89, 0.86 and 0.87 for the three wavelengths of 930, 1121 and 1345 nm, respectively, using near-infrared hyperspectral imaging analysis. The superiority of the imaging techniques compared to traditional analyses methods is that they allow displaying distribution of the analysed properties. On-line inspection of poultry carcasses is also feasible.

**Other Foodstuff and Applications**

Mokhtar et al. (2011) studied the in-line detection of crumbling, in-homogeneity, unevenness in size, cracks and glutinous on the surface of pasta (stick together). Flat LED light source, a CCD camera were used and a production line monitoring system was developed accepting process analytical technology basis using a microcontroller of camera. The error in determination of stickiness, crumples and unevenness in size were 1%, 5% and 13%, respectively. It was also noted that being glutinous was easily detected. Abdullah et al. (2000) investigated the possibilities of classification of muffins with respect to their colour. A classification algorithm used for separating dark from light samples using pre-graded and ungraded muffins. Classification correction rates were 96% of pre-graded and 79% of ungraded muffins when compared with visual inspection.

A computer vision system, developed by Kılıç et al. (2007) examined the classification of beans using artificial neural networks. Size and colour were the parameters for the application. Firstly, length and width were measured both by a calliper and the system. High correlation ($r=0.984$ and $0.971$ for length and width,
respectively) was determined between the results. Secondly, the classification of beans into five classes was performed by human inspector and system. There were rate of correction of classify such that 99.3% of white beans, 93.3% of yellow–green damaged beans, 69.1% of black damaged beans, 74.5% of low damaged beans and 93.8% of highly damaged beans sorted correctly. The overall correct classification rate obtained was 90.6%.

Shafiee et al. (2014) developed a system for characterization of honey based on colour. Estimation of ash content, antioxidant activity and total phenolic content were intended using artificial neural networks. The correlation coefficients of predictions were 0.99, 0.98 and 0.87 for ash content, antioxidant activity and total phenolic content, respectively. They were found to be very promising. These results showed that such a system could effectively applicable for the industry.

The computer imaging systems can also be implemented in dairy industry. For instance a study was conducted by Fu et al. (2014) to determine melamine contamination in milk powder by a near-infrared hyperspectral imaging system. Three different spectral similarity analyses were performed to identify melamine particles in various concentrations. The study results revealed that system could detect melamine at very low concentrations (from 0.02% to 1%) in milk powder and authors also reported their providences that the method had potential to identify some kind of chemicals. Huang et al. (2014) aimed to obtain total chemical information of a milk powder mixed with three different components (ZnSO₄, lactose and melamine). In this study near-infrared micro-imaging system was employed to acquire information and distribution of three target components. The correlation coefficients for detection of melamine in various concentrations (30%, 10% and 5%, w/w) were all over 0.9. The success of determination was decreased and being less than 0.61 with reducing melamine concentration (1%, w/w), although this compound was still detectable. Encouraging results were also obtained for ZnSO₄ and lactose. In a previous study Eskelinen et al. (2007) investigated the possibility of quality and process control of Swiss cheese. 3D ultrasound imaging technique was conducted in order to acquire images. Cheese-eyes (gas holes), cracks and bulk cheese matrix could be distinguished with high success.

One of the newest technology was studied by Lee, Lee (2014) is based on using terahertz (THz) imaging. The inspection of foreign materials like metal objects in food products is commonly performed by X-ray radiation or ultrasonic detectors. If the food contains active compounds such as lactobacillus or fungus, there are concerns of radiation damage and residual radiation in specific materials. On the other hand, being free from radiation hazards, ultrasonic technology, especially low photon energy, is convenient for food applications. By improving resolution quality with a high-power gyrotron, a THz detector, and optical mirror system, a 0.8 mm resolution was achieved using a 0.4 THz transmission image. Consequently, it was demonstrated that it is possible to inspect foreign objects even in packaged food products using this technique in a non-destructive and radiation-free way unless the packaging material contains metal elements like aluminium (Lee, Lee, 2014).

Imaging systems are not used just for food and agriculture products and also used food processing surface. In their study Jun et al. (2009) tried to determine microbial biofilms on the surfaces in contact with food and agriculture products. They used a hyperspectral fluorescence imaging system. Maximum emission was identified at approximately 480 nm. The results indicated that it is possible to detect presence of microbial biofilm on the different surfaces such as stainless steel, polypropylene, formica and granite.

Conclusions
This paper represents basic knowledge about computer image analysis systems used in the food industry including general step of imaging processing and analyses with some examples on applications.

Computer imaging systems make possible to estimate some internal and external quality parameters and to enhance food and agriculture products safety and quality. Automated, rapid, hygienic, non-destructive and objective inspection can be accomplished with these systems. Having wide range of application field, being cheaper and usable in-line processes and their flexibilities make imaging systems more attractive. It seems that further improvements in technology and continued development in computer imaging technology will bring out higher implementations understanding of these systems and allow providing growing needs of the food industry.

References


INTRODUCTION

Calendula (Calendula officinalis L.) belongs to the Asteraceae family. Depending on variety and culture, the plants grow up to 60 cm. Flowers color varies from yellow to orange and they bloom from June until late autumn (Kemper, 1999; Mills, 1999). Calendula is a native of southern Europe but on commercial scale it is cultivated in all around the world. Calendula is a medicinal plant and its flowers are used as important ingredient of pharmaceutical, cosmetic and food production (Cromack, Smith, 1998; Rubine, Enina, 2004). Most calendula research has been focused on extraction and pharmaceutical properties of bioactive compounds from flowers. The name carotenoids, is derived from the fact that they constitute the major pigment in the carrot root, Daucus carota, are undoubtedly among the most widespread and important pigments in living organism. Carotenoids are the pigments responsible for the colors of many plants (Eldahshan, Singab, 2013). The yellow and orange color of flower petals is mostly due to the carotenoids and the shade depends on the quantity and composition of pigments. Carotenoids are known as biologically active compounds with multiple applications in therapy. It is important to humans as precursors of vitamin A and retinoids. Major factors that impact differences in the amount of total carotenoids in calendula flowers is reported to be the plant variety, colour of the ligulate and tubular florets, the place of cultivation and time of harvesting. Nowadays many important carotenoids are used as pigments – food colorants in the food industry. Carotenoids composition in calendula flowers has been recently begun to explore and in future the scientific research on calendula will be increased. In this review, existing information of carotenoids composition in calendula flowers depending of variety are explored, as well as carotenoids multi-activity and usage of calendula flowers are highlight.

CHARACTERIZATION AND HEALTH APPLICATIONS OF CAROTENOIDS

Carotenoids are tetraterpenoids synthesized in plants and microorganisms. They can also be found in many animal species and are important colorants in birds, insects, fish and crustaceans. However, animals and humans cannot synthesize carotenoids and their level in body depends on dietary supply (Britton, 1995; Olson, Krinsky, 1995). Up to now, more than 750 different carotenoids have been isolated from natural sources and new carotenoids continue to be identified (Ramawat, Merillon, 2013). However, only about 40 carotenoids can be absorbed, metabolized, and used in human body. That number is reduced to 6 if the carotenoid profile that is usually detected in human blood plasma is considered. This group includes α- and β-carotene, lycopene, β-cryptoxanthin, zeaxanthin and lutein, which are regularly presented in food list of human diet (Britton, Khachik, 2009).

From the chemical point of view, structurally carotenoids are divided into two major groups: carotenes and xanthophylls. Firstly, carotenes or exclusively hydrocarbon carotenoids only composed of carbon and hydrogen atoms, examples of these compounds are α-carotene, β-carotene, lycopene, etc. Secondly, xanthophylls are known as oxygenated hydrocarbon derivatives that contain at least one oxygen function such as hydroxyl, keto, epoxy, methoxy or carboxylic acid groups. Examples of these compounds are a zeaxanthin and lutein (hydroxy), spirilloxanthin (methoxy) and antheraxanthin (epoxy), etc. (Ramawat, Merillon, 2013).

Many research results shows, that the consumption of a diet rich in carotenoids has been epidemiologically correlated with a lower risk for several diseases (Faulks et al., 2005). Carotenoids are considered as important to humans as precursors of vitamin A and retinoids. The major provitamin A carotenoids in Western diet is β-carotene, but also α-carotene, and β-cryptoxanthin contribute to vitamin A supply and may prevent vitamin A deficiency (Olson, Krinsky, 1995; Het Hof et al., 2000). The importance of carotenoids in food goes beyond as natural pigments and biological functions and actions have increasingly been attributed to them through their multitude of biological activities. Carotenoids are known to possess a wide array of different biological activities such as antioxidant, antitumoral, anti-inflammatory, photoprotective, anti-atherogenic, anti-atherosclerotic, and anti-atherogenic properties. Moreover, it is also known that some carotenoids may act as precursors of vitamin A and retinoids. This is likely the major reason why carotenoids are so important in human nutrition.
to these pigments (Faulks et al., 2005). Carotenoids in tissues reflect food choices too (Rock, 1997). Epidemiologic evidence links higher carotenoid intakes and tissue concentrations with reduced cancer and cardiovascular disease risk (Rock, 1997; Mayne, 1996). There is convincing evidence that carotenoids are important components of the antioxidant network (Rock, 1997). Photooxidative damage is suggested to be involved in the pathobiocemistry of several diseases affecting the skin and the eye, and carotenoids may protect light-exposed tissues.

Lutein and zeaxanthin are the predominant carotenoids of the retina and are considered to act as photoprotectants preventing retinal degeneration. The unique distribution, localization and high levels of both carotenoids within the macula lutea as well as their physicochemical properties make them suitable candidates for photoprotection (Laurence et al., 2000; Stahl, Sies, 2005; Richar, 2013). There are few reports on the toxicity studies of lutein and histopathological analysis of the organs supported the nontoxicity of lutein and its ester form (Harikumar et al., 2008).

β-carotene is one of the most abundant carotenoids in the human body. Comparing to carotenoids that can be metabolized into vitamin A, β-carotene has the highest provitamin A activity (Krinsky, Johnson, 2005). According to the researches, β-carotene is widely used as an oral sun protectant for the prevention of sunburn and has been shown to be effective either alone or in combination with other carotenoids or antioxidant vitamins (Sies, Stahl, 2004; Stahl, Sies, 2005; Berneburg, Krutmann, 2000; Stahl et al., 2000).

Protective effects are also achieved with a diet rich in lycopene. Many studies suggest that eating lycopene rich foods or having high lycopene levels in the body may be linked to reduced risk of cancer, heart disease, and age-related eye disorders. However, measures of lycopene intake have been based mainly on eating tomatoes and other products with high lycopene content, not on the use of lycopene supplements (Krinsky, Johnson, 2005; Rock, 1997; Sies, Stahl, 2004).

**Carotenoids in calendula flowers**

The several investigations of carotenoids compositions of calendula petals have been reported although the researches on it has been recently begun to investigate. Some report refers to qualitative aspects as separation and identification of carotenoids, and others to quantitative determination as total carotenoids. According to the researches, there are found out that calendula accumulates large amounts of different carotenoids in its flowers.

Bako et al. (2002) have studied the composition of carotenoids in calendula petals that were obtained from commercial garden in Pécs (southern Hungary) and found about 18 different types of carotenoids: neoxanthin, (9 Z)-neoxanthin, violaxanthin, luteoxanthin, auroxanthin, (9 Z)-violaxanthin, flavoxanthin, mutatoxanthin, (9 Z)-antheraxanthin, lutein, (9/9 Z)-lutein, (13/13 Z)-lutein, α-carotene, β-carotene. Kishimoto et al. (2005) were analyzed three orange flowered cultivars (‘Alice Orange’, ‘Orange Star’ and ‘Orange Zem’) and three yellow flowered cultivars (‘Alice Yellow’, ‘Gold Star’ and ‘Golden Zem’) of calendula in their researches, grown in Tsukuba, Japan. They have identified 19 different carotenoids in their researches, such as, (8 R’)-luteoxanthin, lutein-5,6-epoxide, flavoxanthin, (8R/8’ R)-auroxanthin, (9 Z)-lutein-5,6-epoxide, lutein, antheraxanthin, (9Z)-lutein, (5 Z/9 Z)-rubixanthin, α-carotene, β-carotene, (5 Z)-rubixanthin, δ-carotene, (5Z9Z/5 Z/9 Z)-lycopene, γ-carotene, (5 Z)-γ-carotene, (5Z9Z/5Z)-lycopene, (5Z/9Z)-lycopene, all-E-lycopene. Two carotenoids of them (5 Z)-γ-carotene and (5 Z/9 Z)-rubixanthin) have never been before identified. However, they have not identified numerous of carotenoids that have been found in by other researcher from other countries. From these results we can find out that the place of cultivation impact composition of different carotenoids in calendula flowers.

Carotenoids are generally responsible for petal colors in the yellow to red range and the wide range of petal colors in various varieties of calendula originates mainly from combinations of these pigments. Verghese (1998) has reported that marigold (Tagetes erecta) flower petals are a significant source of the xanthophyll and have a much higher concentration of this pigment compared to other plant materials. Moehs et al. (2001) have reported that in marigold (Tagetes erecta), the differences in petal color from pale-yellow to orange-red are caused by different levels of accumulation of yellow carotenoid lutein.

Kishimoto et al. (2005) have shown that distinctive differences in carotenoids content (characterized by HPLC chromatograms) were found between orange flowered varieties and yellow flowered varieties. In all, 19 carotenoids where observed in orange flowered varieties, and of these, 10 carotenoids were detected only in orange flowered varieties. They have found out that yellow colored petals don’t contain (5 Z/9 Z)-rubixanthin, α-carotene, (5 Z)-rubixanthin, δ-carotene, (5Z9Z/5 Z/9 Z)-lycopene, γ-carotene, (5 Z)-γ-carotene, (5Z9Z/5Z)-lycopene, (5Z/9Z)-lycopene, all-E-lycopene. Therefore we can consider that these carotenoids are responsible for the orange color formation of the petals. The similar results have shown Pinta et al. (2003) in their researches of four calendula cultivars (‘Alice Orange’ and ‘Radio Extra Selected’) and two yellow flowered (‘Bonbon Abricot’ and ‘Double EstrelJaune’). They have found out that the composition of different carotenoids increases with color intensity, the dark orange variety being the richest one. The dark flowered variety (‘Double Estrel Orange’) is the richest in rubixanthin, lycopene and γ-carotene, but yellow flowered variety (‘Bonbon Abricot’) contains important amount of β-carotene, but very low amount of γ-carotene and it is especially low in lycopene. Kishimoto et al. (2007) have reported, that
orange and yellow flowered cultivars of *O. ecklonis* (*Compositae* family) showed the same composition, the proportions of three reddish carotenoids – 5′Z-γ-carotene, 5Z/9Z/5′Z-lycopene, and lycopene – were higher in the orange flowered cultivar. In general, there is found out that orange flowered cultivars of calendula accumulate more reddish carotenoids than yellow flowered varieties (Kishimoto, Ohmiya, 2009). The color intensity of orange flowered varieties is primarily due to lycopene, which is absent from yellow flowered varieties. Lutein and β-carotene were the most abundant carotenoids in the Italian type of calendula, grown in Ravenna, northern Italy (Piccaglia et al., 1999).

More reports refer only to quantitative determination as total carotenoids. The major factors that impact differences in the amount of total carotenoids in calendula flowers is reported to be the plant variety, color of the ligulate and tubular florets, the place of cultivation and time of harvesting (Bako et al. 2002; Kishimoto et al. 2005; Kishimoto et al. 2007; Kishimoto, Ohmiya, 2009; Pintea et al., 2003; Raal et al., 2009), as well as, environmental factors, such as soil mineral composition, temperature, oxygen and light (Piccaglia et al., 1999).

A wide range of calendula varieties were analyzed in Estonia. Raal et al. (2009) and Toom et al. (2007) have found out that the total carotenoids content varied in the investigated 42 samples from 200 to 3510 mg 100 g⁻¹ dried flowers. The highest total carotenoids content (3510 mg 100 g⁻¹) was found in brownish-yellow flowered variety ‘Golden Emperor’ (Table 1.). The lowest sum of pigments was found in the variety ‘Touch of Buff’ (200 mg 100 g⁻¹), with a very unconventional combination of flowers color – its tubular florets were sorrel, but the ligulate ones were maroon in the top and cream colored in the bottom. In their studies, they have observed that usually canary yellow, carnation-yellow and yellow flowered cultivars contain total carotenoids of less than 1000 mg 100 g⁻¹ while cultivars with orange flowers contain pigments between 1000–2000 mg 100 g⁻¹ or even more. Pintea et al. (2003) have found the highest total carotenoids content in fresh flowers of orange flowered cultivar ‘Double Esterel Orange’ with a total content 276 mg 100 g⁻¹, but lowest in yellow-orange flowered cultivar ‘Bonbon Abricot’ – 48.2 mg 100 g⁻¹. Also Kishimoto et al. (2007) have found differences in total carotenoids content between orange and yellow flowered cultivars.

In general, that the richest in total carotenoids content are brownish-yellow and orange flowered calendula varieties. The total carotenoids content also depends on region of cultivation. The same variety grown in different regions has shown different content of total carotenoids. For example, variety ‘Fiesta Gitana’, grown in Central Estonia (Järvamaa) has shown 1060 mg 100 g⁻¹, but towards to the south Estonia (Viljandimaa) total carotenoids content has increased to 1440.0 mg 100 g⁻¹ (Raal et al., 2009). Pintea et al. (2003) have reported that the values of quantitative determinations are very different from one variety to another. Anyhow, the interconnection between calendula varieties and content of total carotenoids is not satisfactorily clear and therefore there more researches in future are needed.

### Table 1

**Amount of total carotenoids in flowers of calendula (*Calendula officinalis* L.) varieties**

<table>
<thead>
<tr>
<th>Variety (color of flowers)</th>
<th>Carotenoids, mg 100 g⁻¹</th>
<th>Place of cultivation (region, country)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fresh flowers</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alice Orange (orange)</td>
<td>170*</td>
<td>Tsukuba, Ibaraki, Japan</td>
</tr>
<tr>
<td>Alice Yellow (yellow)</td>
<td>125*</td>
<td>Tsukuba, Ibaraki, Japan</td>
</tr>
<tr>
<td>Bonbon Abricot (yellow-orange)</td>
<td>48**</td>
<td>Cluj-Napoca, Romania</td>
</tr>
<tr>
<td>Double EsterelJaune (lemon yellow)</td>
<td>97**</td>
<td>Cluj-Napoca, Romania</td>
</tr>
<tr>
<td>Double Esterel Orange (dark orange)</td>
<td>276**</td>
<td>Cluj-Napoca, Romania</td>
</tr>
<tr>
<td>Gold Star (yellow)</td>
<td>126*</td>
<td>Tsukuba, Ibaraki, Japan</td>
</tr>
<tr>
<td>Golden Gem (yellow)</td>
<td>118*</td>
<td>Tsukuba, Ibaraki, Japan</td>
</tr>
<tr>
<td>Orange Gem (orange)</td>
<td>107*</td>
<td>Tsukuba, Ibaraki, Japan</td>
</tr>
<tr>
<td>Orange Star (orange)</td>
<td>145*</td>
<td>Tsukuba, Ibaraki, Japan</td>
</tr>
<tr>
<td>Ponpon Orange (orange)</td>
<td>119*</td>
<td>Tsukuba, Ibaraki, Japan</td>
</tr>
<tr>
<td>Ponpon Yellow (yellow)</td>
<td>109*</td>
<td>Tsukuba, Ibaraki, Japan</td>
</tr>
<tr>
<td>Radio Extra Selected (orange)</td>
<td>112**</td>
<td>Cluj-Napoca, Romania</td>
</tr>
<tr>
<td><strong>Dried flowers</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apricot Beauty (dark orange-canary yellow)</td>
<td>530***</td>
<td>Järvamaa, Central Estonia</td>
</tr>
<tr>
<td>Apricot Beauty (yellow, top orange)</td>
<td>430***</td>
<td>Viljandimaa, Southern Estonia</td>
</tr>
<tr>
<td>Art Schades (canary yellow-light-orange)</td>
<td>1780***</td>
<td>Järvamaa, Central Estonia</td>
</tr>
<tr>
<td>Art Schades (yellow-orange)</td>
<td>840***</td>
<td>Viljandimaa, Southern Estonia</td>
</tr>
<tr>
<td>Balls Orange (orange)</td>
<td>2000–2240***</td>
<td>Viljandimaa, Southern Estonia</td>
</tr>
<tr>
<td>Balls Orange (orange)</td>
<td>1150***</td>
<td>Järvamaa, Central Estonia</td>
</tr>
<tr>
<td>Balls Supreme (orange)</td>
<td>1750***</td>
<td>Viljandimaa, Southern Estonia</td>
</tr>
<tr>
<td>Cremeweiss (canary yellow)</td>
<td>370***</td>
<td>Viljandimaa, Southern Estonia</td>
</tr>
<tr>
<td>Cremeweiss (canary yellow)</td>
<td>270***</td>
<td>Pärnumaa, South-Western Estonia</td>
</tr>
</tbody>
</table>
Usage of calendula flowers

Calendula is with huge potential in medicine, food and cosmetic production (Rubine, Enina, 2004; Saussere, Kampuss, 2014). It is used as therapeutically plant from ancient time. In human medicine calendula flowers are used in tea, infusions, tinctures, etc. (Loranty et al., 2010; Rubine, Enina, 2004). It was efficient for liver illness, cramps, ulcers, jaundice, hemorrhoids, internal healing, skin cancer treatment (Khalid, Jaime, 2012; Muley et al., 2009; Preethi et al., 2009) and also with wide spectra of action, such as, choleretic, antispasmodic, bactericidal, anticancer, wounds, frostbite, burns, acne. Calendula is applied in a form of lotion for skin illness, ulcerations, frostbites, burns, fungus (Jorge et al., 1996; Preethi et al., 2009). Extracts derived from dried flowers inhibit the replication of HIV-1 (Kalvatchev et al., 1997). In veterinary medicine the raw material of calendula flowers is used for the preparation of different tinctures (Grela, Sembratowicz, 1998).

Dried and fresh flowers of calendula are widely used as food. In many parts of the world people continues to prepare food with old traditions. More and more the assortments of foodstuffs produced begin to be markedly extended with edible flowers. And these flowers could be potential rich resources of natural antioxidants and carotenoids and could be developed into functional foods, which increases and improves the appearance, taste and aesthetic value of these foodstuffs (Tanji, Nassif, 1995; Mlcek et al., 2011). According to the researches, calendula flowers accumulate high amount of different carotenoids. Since the content of these compounds are not very high in the majority of food stuffs (Mlcek et al., 2011), calendula flowers as edible flowers could be one of the sources of valuable substances. Fresh flowers are served as garnish and trimming of various meals and cold buffet food, and petals are used to decorate salads, sweet meals, fruit and ice-cream sundaes, drinks, etc. In addition to the esthetic appearance they also correspond to specific taste and smell of served food (Rubine, Enina, 2004; Scherf, 2004). It has long been shown in human studies that fat enhances the absorption of carotenoids (Castenniller, West, 1998). In recent studies, greater absorption of carotenoids was observed when salad was consumed...
with full-fat than with reduced-fat salad dressing (Brown et al., 2004; Rodriguez-Amaya, 2010). Calendula flowers can be also dried, soaked in alcohol or sugar, frozen either directly or in form of ice cubes, added in to cocktails, etc. The freeze drying is not cost effective but preserves the original appearance, color, shape, and gloss of flowers (Mlcek et al., 2011; Mohammad, Kashani, 2012; Rubine, Eniña, 2004). At the beginning of the food industry, pigments – natural of synthetic, were used to give an attractive appearance, perception of freshness, taste, and quality of food. Today, natural colorants are emerging globally due to the perception of its safer and eco-friendly nature (Pratheesh et al., 2009; Abbey et al., 2014). Carotenoids pigments are of interest to the food scientists, nutritionists and food industries due to their positive impact on human health and their economic benefits. Carotenoids are responsible for the attractive color of many plant foods, which is perhaps the first attribute that consumers assess when determining the quality and appearance of a product, and therefore conditions of its acceptability (Fernandez-Garcia et al., 2012).

Conclusions
Nowadays many important carotenoids are used as pigments – food colorants in the food industry. Calendula accumulates large amount of different carotenoids in its flowers, therefore its promising plant for condiment of food production, as well as – food colorants in the food industry. Nowadays, a trend towards researches of calendula represents a challenge for food manufactures, because of its widely therapeutically applications, and diet rich in carotenoids represents lower risk for several diseases. The addition of many colors to healthy food and food products may become superfluous in the future. In collaboration of specialists of agriculture, bioengineering and food science it is possible to develop innovative techniques to produce foods that could be potential rich of natural carotenoids and to find out good colorants and to improve retention of total carotenoids pigments, as well as flavors and nutrients in cooked food.

References


REVIEW

HIGH-OXYGEN MODIFIED ATMOSPHERE PACKAGING OF ORGANIC MEATS?

Friedrich-Karl Lücke, Sonja Schreiber

Department Nutritional, Food and Consumer Sciences, Fulda University of Applied Sciences, Marquardstr. 35, 36039 Fulda, Germany, e-mail: friedrich-karl.luecke@hs.fulda.de

Abstract

In the last few years, it became widespread to package fresh meat for retail under modified atmosphere containing 70–80% oxygen (“high O2-MAP”). Oxygen preserves the bright-red colour of oxymyoglobin while CO₂ (20–30%) inhibits obligatory aerobic bacteria such as psychrotrophic Pseudomonas spp., the common spoilage agents for unpackaged fresh meat. On the other hand, high oxygen partial pressure has been shown to cause some oxidative changes in the meat, resulting in sensory deviations. Use of high O2-MAP is also legally permitted for the packaging of organic meats. This may be regarded as being in conflict with the principle of “processing food with care” as stipulated by Article 6 of the Regulation (EU) 834/2007 on organic production and labelling of organic products. Therefore, the aim of our research was to provide information to decision-makers in the organic food chain on the necessity of high O2-MAP for organic meats. Literature data from market research show that even consumers of organic food seek “convenience” in purchasing and preparing it. Hence, of the major associations of organic agriculture in Germany, only Demeter e. V. prohibits the use of high O2-MAP. Literature data also indicate that undesirable changes of the meat could be delayed by reducing the oxygen content in the packages to about 40–50%, with only a slight reduction of shelf life. In our view, this would be a good compromise between “convenience” and eating quality.

Keywords: meat, packaging, modified atmosphere, organic.

Introduction

In various countries, there is a major trend that consumers tend to reduce the time spend for shopping and for preparing food in their households. The socio-economic reasons for it have been summarized by Schulze and Spiller (2008a) for Germany. As a consequence, an increasing percentage of food is sold in retail packages rather than “over the counter”. This also applies for fresh meat. From 2000 to 2005, the market share of pre-packaged red meat increased from 23 to 42% (Schulze, Spiller, 2008b). In Europe, the percentage is similar (Belcher, 2006). The Fraunhofer-IVV Institute (Anonymous, 2013a) mentioned a market share for pre-packaged fresh meat (chilled or frozen) of 53% in 2010. Recent data are available for processed meats (hams, sausages) in Germany: From 2005 to 2012, the market share of pre-packaged products (excluding canned meats) increased from 62.3 to 65.3% (Anonymous, 2013b).

Appearance is a critical factor affecting buying decisions at the point of sale. The chemistry of meat colour has been reviewed by Manchini and Hunt (2005). A bright-red colour of oxymyoglobin is regarded as an indicator of freshness of meat, in particular, beef and pork (Carpenter et al., 2001; Becker et al., 2000; Robbins et al., 2003). At sufficient levels of oxygen, myoglobin is kept in this form whereas low oxygen partial pressure accelerates the formation of brownish metmyoglobin. Total exclusion of oxygen is difficult to achieve, and the resulting dark-red colour of myoglobin is less attractive, especially for pork. Hence, most pre-packaged fresh meat is displayed under oxygen-enriched atmospheres (“high O2-MAP”). To avoid growth of aerobic spoilage bacteria such as Pseudomonas spp., carbon dioxide is included in the package at levels of 20–30%. As a “rule of thumb”, high-O2-MAP of fresh meat may double the time until spoilage by micro-organisms (see Sun, Holley, 2012).

It is common practice to package fresh meat under 70–80% oxygen (“high O2-MAP”) plus 20–30% CO₂. Such high oxygen levels, however, have been shown to cause some oxidative changes in the meat, resulting in sensory deviations. Such changes occur in lipids and proteins. Thus, it may be argued that the consumer is misled because the meat looks fresher than it actually is. Accordingly, packaging meat in high O2-MAP has been criticised by consumer organisations (Anonymous, 2013c).

According to Article 6 c and d of the Regulation (EC) 834/2007 on organic production and labelling of organic products (Anonymous, 2007), substances and processing methods should be excluded “that might be misleading regarding the true nature of the product”, and the processing of food should be done “with care”, preferably with the use of biological, mechanical and physical methods. Nevertheless, high O2-MAP is also used for the packaging of organic meats.

Therefore, the aim of our research was to provide information to decision-makers in the organic food chain on the necessity of high O2-MAP for organic meats, with focus on red meats (beef, pork).

Approach

We searched the literature on data about effects of high-O2 MAP on meat quality, analysed the legal situation in the European Union, and interviewed representatives of the German “organic food sector” on the compatibility of high-O2 MAP with the requirements of organic food processing.
Results and Discussion

1. Effect of oxygen levels in MAP on meat quality parameters

Colour

Table 1 summarizes data on atmospheres in MAP giving colour stability equal to high-\(O_2\) MAP (70–80\% \(O_2\)). It may be seen that the oxygen content in the package may be reduced to about 40–50\% without major effects on colour and shelf life.

<table>
<thead>
<tr>
<th>Product</th>
<th>Gas composition(^1)</th>
<th>Storage (^2)</th>
<th>Ref.(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(O_2)</td>
<td>(CO_2)</td>
<td>°C</td>
</tr>
<tr>
<td>Beef steak</td>
<td>55</td>
<td>45</td>
<td>2</td>
</tr>
<tr>
<td>Beef steak</td>
<td>40</td>
<td>20</td>
<td>4</td>
</tr>
<tr>
<td>Beef steak(^2)</td>
<td>60</td>
<td>40</td>
<td>4</td>
</tr>
<tr>
<td>Ground beef</td>
<td>50</td>
<td>30</td>
<td>4</td>
</tr>
<tr>
<td>Pork chop</td>
<td>35</td>
<td>20</td>
<td>4</td>
</tr>
<tr>
<td>Pork retail cuts</td>
<td>20</td>
<td>20</td>
<td>2</td>
</tr>
</tbody>
</table>

\(^1\) balance: nitrogen.
\(^2\) aged 7 days, frozen and thawed before storage
\(^3\) (1) Jakobsen, Bertelsen, 2000; (2) Zakrys et al., 2009; (3) Zakrys-Waliwander et al., 2011; (4) Esmer et al., 2012; (5) Esmer et al., 2011; (6) Zhang, Sundar, 2005; (7) Nitsch, 2012

Sensory deviations due to oxidative changes

Sensory deviations of meat packaged under high \(O_2\) levels were frequently reported. Deficiencies include lack of meat aroma, rancidity, excess warmed-over flavour, as well as toughness and lack of juiciness (Lautenschläger, Müller, 2006; Dederer et al., 2014). In high-\(O_2\) MAP, the rate of lipid oxidation is higher, as indicated by the concentrations of thiobarbituric acid-reactive substances (Jakobsen, Bertelsen 2000; John et al., 2004; Seyfert et al., 2004; Clausen et al., 2009; Kim et al., 2010). However, at least for beef steaks and ground beef, considerable less oxidative changes could be achieved by reducing the oxygen level in MAP to about 50\% (Table 2).

A lack of tenderness of high-\(O_2\) MAP meat is mainly due to cross-linking of myosin molecules (Lund et al., 2011; Kim et al., 2010). However, this effect could be minimized by reducing the oxygen level in MAP to 40\% (Zakrys et al., 2009).

Table 1. Gas atmospheres giving a meat colour similar to high-\(O_2\) MAP (70–80\% \(O_2\))

<table>
<thead>
<tr>
<th>Product</th>
<th>Gas composition(^1)</th>
<th>Storage (^2)</th>
<th>Ref.(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(O_2)</td>
<td>(CO_2)</td>
<td>°C</td>
</tr>
<tr>
<td>Beef steak</td>
<td>50</td>
<td>30</td>
<td>4</td>
</tr>
<tr>
<td>Ground beef</td>
<td>50</td>
<td>30</td>
<td>4</td>
</tr>
<tr>
<td>Pork chop</td>
<td>25</td>
<td>20</td>
<td>4</td>
</tr>
</tbody>
</table>

\(^1\) balance: nitrogen.
\(^2\) aged 7 days, frozen and thawed before storage
\(^3\) (1) Zakrys-Waliwander et al., 2011; (2) Esmer et al., 2011; (3) Zhang, Sundar, 2005.

Cholesterol oxidation

During storage of meat in high-\(O_2\) MAP, cholesterol oxidation products (COP) are formed, in particular, 7\(β\)-hydroxy- 7\(α\)-hydroxy-, 7-keto- and \(β\)-epoxycholesterol (Ferioli et al., 2008; Boselli et al., 2009). In beef slices packaged in MAP containing 32\% \(O_2\) and exposed to light, levels were about twice as high as in meat packed in air (Boselli et al., 2009). There is some evidence from in-vitro experiments and from animal studies that uptake of these COP increases the risk of atherosclerosis (Anonymous, 2010a). On the other hand, the type of meat, the length of storage and the cooking process also affect the levels of COP, and it was concluded that meat stored in high-\(O_2\) MAP contributes only little to the overall uptake of COP (Anonymous, 2010a). However, in view of the scarcity of data, it is advisable to minimize the formation of COP during meat storage and processing.

Premature browning

Consumers normally use greyish-brownish discoloration of meat as an indicator for doneness during cooking. Indeed, times and temperatures to achieve this are similar to those required for inactivation of pathogens such as salmonellae, shigatoxin-forming *Escherichia coli* (STEC) and *Campylobacter*. Various authors (Hunt et al., 1999; Seyfert et al., 2004; Clausen et al., 2009; Rasvoll et al., 2014) observed that meat packaged under high-\(O_2\) atmospheres turn grey/brownish at lower cooking temperatures. This is due to the fact that oxymyoglobin is less stable to heat than myoglobin. “Premature browning” may result in a health hazard, especially to the consumer of hamburgers and similar cooked minced meats. For example, hamburgers looked “done” at internal temperature of about 60 °C. Stopping the cooking process at this stage will not inactivate STEC to a sufficient degree. In a survey, most consumers judge doneness by colour and preferred the batch with premature browning (Rasvoll et al., 2014). Reduction of oxygen levels in MAP to
50% did not completely eliminate this problem (Clausen et al., 2009).

2. Consumer attitudes and expectations

In response to open questions, German consumers (n=991) mention “freshness” as the main quality criterion for meat, followed by price and origin (Branscheid, 2008). “Colour” was mentioned by only 18% of all respondents, and by 23% of respondents stating that they eat meats daily. These results are in contrast to data clearly showing the major role of meat colour in buying decision (Becker et al., 2000; Robbins et al., 2003; Carpenter et al., 2001). Moreover, in open questions, only few consumers mentioned “best-before date” as a quality criterion. Therefore, it is plausible to assume that consumers trust their senses when buying meat and appearance (of the meat and the point of sale) is the main criterion.

In contrast, the best-before date was mentioned as an important criterion for their buying decisions by 87% of consumers participating in the German National Nutrition Survey II (Anonymous, 2008a). The results are based on questionnaires that eliminate most of the bias introduced by face-to-face or even telephone interviews and may reflect the reality more closely.

In May 2013, 1 002 German consumers were asked (by telephone interviews) about their attitude towards organic food (Anonymous, 2013d). Consumers regularly buying organic food mentioned regional origin (87%), animal welfare (85%), and absence of residues (83%) as the most important reasons. This confirms the importance of altruistic motivations, as also stressed by Padilla Bravo et al. (2013) on the basis of data from German National Nutrition Survey II. Of various parameters affecting buying decisions, “optimal freshness and quality” ranked first. 32% of the interviewees stated that they frequently (or even exclusively) buy organic meats. Consumers have more trust in the original producers of organic food, and in specialized shops but in contrast, they buy most organic food from supermarkets, and even from discounters, which are perceived as even less trustful. This indicates that, along with price, “convenience” in shopping is a major criterion even for the consumer of organic food (Padilla Bravo et al., 2013). However, many consumers may not always be ready to admit this.

3. Legal situation for conventional and organic meat

It is generally prohibited to mislead the consumer by insufficient or misleading information (see Article 7 of Regulation (EU) no. 1169/2011;Anonymous, 2011). Hence, the key question is whether or not the consumer mistakes “old” meat for fresh. To date, it is compulsory to label the use of “protective atmospheres” but not to indicate the composition of gas. In a document published by a working group of experts from the German official food control, it was stated that offering unpackaged meat treated with hyperbaric oxygen in order to restore its bright-red colour is only permitted if the consumer is appropriately informed about this treatment (Möllers et al., 2007).

Oxygen is a permitted food additive in the European Union. It is also listed in Annex VIII Section A of Regulation (EC) No. 889/2008 (Anonymous, 2008b) as a permitted additive for organic foods. German non-governmental associations for organic food generally prohibit the use of high-pressure oxygen treatment to restore the bright-red meat colour. Of the German associations, Bioland e.V. (Anonymous, 2010b) and Naturland e.V. (Anonymous, 2013e) permit high-O₂ MAP packaging of meat, arguing that O₂ only affects the colour of the surface. In contrast, Demeter e.V. approves only nitrogen and CO₂ as packaging gases (Anonymous, 2013f).

4. Alternatives to high-O₂ MAP

The available data indicate that reducing the oxygen level in MAP to about 40–50% still sufficiently stabilizes meat colour while reducing sensory deficiencies caused by oxidative processes. Other packaging methods for fresh meat have been reviewed by McMillin (2008) and O’Sullivan and Kerry (2010). Packaging in vacuum-skin packages may reduce oxygen levels so much that metmyoglobin formation is inhibited, and had a favourable effect on beef colour (Li et al., 2012). However, with many meat cuts (especially from pork), the dark-red colour of myoglobin is less attractive to the customer, and acid formation by lactic acid bacteria on the meat surface during storage may affect the globin moiety and lead to discoloration. The inclusion of carbon monoxide stabilizes the meat colour in the absence of oxygen but spoilage may be masked, and CO is unlikely to ever be approved for organic meat. Fat oxidation in meat packaged in high-O₂ MAP may be retarded in avoiding exposure to UV light (Anonymous, 2013a). Last but not least, it makes no sense to package “white meat” (e. g. many cuts from poultry) under oxygen. This also applies to poultry meat covered with skin.

Conclusions

In summary, using high-O₂ MAP with 70-80% oxygen appears in contradiction to the principle of “careful” processing of organic meat. On the other hand, there is clearly a market demand for fresh organic meats in convenient retail packages. A sound compromise could be to reduce oxygen levels in MAP to about 40-50% or even less.

Acknowledgment

We are grateful to the experts sharing their experience and views with us, and to Alexander Beck and Renate Dylla (Büro Lebensmittelkunde und Qualität, Bad Brückenau) for their advice.
References


REVIEW

PHYTOSTEROLS IN RICE BRAN AND USAGE OF RICE BRAN IN FOOD INDUSTRY

Özgül Özdestan, Tuğçe Erol, Burcu Acar

Department of Food Engineering, Faculty of Engineering, Ege University, Bornova-Izmir, Turkey,
e-mail: ozgul.ozdestan@ege.edu.tr

Abstract

Phytosterols are very important for daily diet that can not be synthesized by human body. Main sources of phytosterols are vegetable oils, seeds, legumes and cereals. Phytosterols prevent cholesterol absorption therefore lower total and LDL cholesterol level in blood. Also plant sterols might protect against certain types of cancer such as colon, breast and prostate. Rice bran is the best source of total lipids and phytosterols based on raw material. Rice bran oil contains very high concentrations of cycloartenol and 24-methylenecycloartenol, which made up over 40% of the total phytosterols. Rice brain contains 12–22% oil, 11–17% protein, 6–14% fiber, 10–15% moisture, and 8–17% ash. Also, It is rich in vitamins, including vitamin E, thiamine, niacin, and minerals like aluminum, calcium, chlorine, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc. Furthermore, presence of antioxidants like tocopherols, tocotrienols, and γ-oryzanol also brighten prospects of rice bran utilization for humans as functional ingredient to reduce the life threatening disorders. To improve quality and nutrition of food product, rice bran can be evaluated as a potential food ingredient. It has been used in food as full-fat, defatted bran, bran oil, and protein concentrates. Rice bran is used by the food industry in the production of baked foods, snacks, crackers, breads, cereals, pastries, pancakes, noodles, muffins, biscuits. In this review, phytosterols found in rice bran which impacts on human health and the usage of rice bran in food industry are discussed in general terms.

Keywords: bioactive compound, cereals, phytosterol, rice bran.

Introduction

Phytosterols (including plant sterols and stanols) can not be synthesized by humans, and all plant sterols and stanols in the human body therefore originate from the diet. The class of sterols known as phytosterols is found mainly in plant cell walls and membranes. Sitosterol, campesterol, and stigmasterol were the major phytosterols in the lipid extracts. Phytosterols have hypocholesterolemic effects (Jong et al., 2003; Jiang and Wang, 2005). They are known to have several bio-active qualities with possible implications for human health (Normen et al., 2002). The difference of phytosterols which are a group of triterpenes from cholesterol is the presence of a double bond within ethyl or methyl groups and within the side chains of them. Phytosterols can be found either in the free form and the esterified form. Phytosterols are classified either as -sterols or -stanols; according to the presence of the double bond within the position Δ5 (Bayrakç, 2013).

Rice bran is the best source of phytosterols based on raw material. Rice bran, a part of the rice kernel that contains pericarp, aleurone, and subaleurone fractions, is a by-product of rice milling. It is estimated that the world annual production of rice bran amounts to 76 million tons. Rice brans, oils, and hulls contain a large number of bioactive compounds, with pigmented brans containing many more bioactive compounds than do white brans (Friedman, 2013).

Because of the increasing interest in relationship between health and food, results increasement of formulation studies about functional products. For this reason, stabilized rice bran or its components have been used as ingredients in various food matrices such as bread (Hu et al., 2009), cookies (Bhanger et al., 2008), pizza (de Delahaye et al., 2005), beverages (Faccin et al., 2009; Jeličić et al., 2008), tuna oil (Chotimarkorn et al., 2008). Rice bran is also used in meat emulsions and batter mixes industrially. In this study we will present brief overviews of phytosterols and selected recent studies on enrichment of some food products with rice bran.

Phytosterols and Chemical Structure

Phytostanols are a fully-saturated subgroup of phytosterols (contain no double bonds). Phytostanols occur in trace levels in many plant species and they occur in high levels in tissues of only in a few cereal species. Phytosterols can be converted to phytostanols by chemical hydrogenation. More than 200 different types of phytosterols have been reported in plant species. In addition to the free form, phytosterols occur as four types of “conjugates,” in which the 3β-OH group is esterified to a fatty acid or a hydroxycinnamic acid, or glycosylated with a hexose (usually glucose) or a 6-fatty-acyl hexose (Moreau et al., 2002). The structures of sitosterol, sitostanol, campesterol and campestanol are shown in Fig. 1 (Gilbert et al., 2005).

Figure 1. Chemical structures of some phytosterols

(Gilbert et al., 2005)
Heath Effects and Resources of Phytosterols
Phytostanol; reducing blood cholesterol levels, as well as their other beneficial health effects, have been known for many years (Quilez et al., 2003). It was recognized in the 1950s that plant sterols lower serum concentrations of cholesterol (Pollak, 1953). Plant sterols might also protect against certain types of cancer such as colon, breast and prostate (Rao, Koratkar, 1997; Awad and Fink, 2000). Phytosterols can be found at widely varying concentrations in the fat-soluble fractions of seed, root stems, branches, leaves and blossoms. They are constituents of both edible and ornamental plants, including herbs, shrubs and trees. As natural constituents of the human diet, phytosterols are naturally found in all food items of plant origin, principally oils, but also pulses and dried fruits. Their content is highest in edible oils, seeds and nuts. The total contents are very variable and range from nearly 8 g kg⁻¹ in corn oil to 0.5 g kg⁻¹ in palm oil, with intermediate levels being found in commonly used oils. Tall oil contains a higher proportion of plant stanols than do vegetable oils. Phytosterols are of products based on vegetable oils. The refining process cited leads to a significant reduction in phytosterols in vegetable oils and it would therefore be very interesting to develop industrial methods which minimize these losses (Taşan et al., 2006). Main sources of phytosterols are wheat bran, wheat germ, durum wheat, rice bran, vegetables oils, seed and legumes (Clifton, 2002; Jiang, Wang, 2005). Germ and bran fraction of cereals, especially rice bran and wheat germ which have high level of oil, are known as best source of phytosterols. Rice bran contains %22.2 of phytosterol (Taşan et al., 2006). The dietary intake of phytosterols among and within different human population varies greatly, depending on the type and amount of plant foods eaten. Although cooking oils, margarine and peanut butter are the main sources of phytosterols in the diet, phytosterols are also consumed in seed, nuts, cereals and legumes (Clifton, 2002). The consumption of phytosterols can range between 170 mg per day in populations eating a Western diet and 360 mg per day in diets rich in vegetable products (Vries et al., 1997). The dietary intake of plant stanols is usually only about 50 mg/day unless the diet is supplement with tall oil, which is derived from conifers and is rich in sitostanol (Gilbert et al., 2005). The normal dietary intake of plant stanols is much less than that of plant sterols.

Rice Bran
Rice is one of the most important staple foods for a large part of human population. Global rice production is 645 million tons and this huge amount of production results large amount of rice by products (Kubglomsong, Threerakulkait, 2014; Al-Okbi et al., 2014). Rice bran is the major by-product generated during the milling processing (Kubglomsong, Threerakulkait, 2014). Brown rice is resulted from dehusking of raw rice and brown rice is covered by bran layers which namely, pericarp, testa and aleurone layers. Bran is obtained by de-branning or polishing process and constitutes 5–10% of brown rice. It’s mainly composed of protein, fiber, oil, vitamins, minerals and starch which come from endosperm during polishing (Al-Okbi et al., 2014). It is also a good source of antioxidants such as polyphenols, tocopherols, tocotrienols and gamma-oryzanol which help in preventing the oxidative damage of body tissues and DNA. Many studies reported that rice bran has cholesterol lowering properties, cardiovascular health benefits and anti-tumor activity (Tuncel et al., 2014a). Table 1 shows the bioactive compounds of rice bran (Friedman, 2013). Jiang and Wang (2005) have studied to determine amount of phytosterols in some cereal by- product (rice bran, wheat bran, oat bran, oat germ, durum wheat, corn fine fiber).

<table>
<thead>
<tr>
<th>Phenolic and cinnamic acids</th>
<th>Anthocyanins, flavonoids</th>
<th>Steroidal compounds</th>
<th>Polymeric carbohydrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeic acid</td>
<td>anthocyanin monomers, dimers, and polymers</td>
<td>acylated steryl glucosides</td>
<td>arabinoxylan</td>
</tr>
<tr>
<td>Coumaric acid</td>
<td>apigenin</td>
<td>cycloartenol ferulate</td>
<td>glucans</td>
</tr>
<tr>
<td>Catechins</td>
<td>cyanidin glucoside</td>
<td>campesterol ferulate</td>
<td>hemicellulose</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>cyanidin rutinoside</td>
<td>24-methylenecycloartenol ferulate</td>
<td></td>
</tr>
<tr>
<td>Gallic acid</td>
<td>epicatechins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyroxybenzoic acid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methoxy cinnamatic acid</td>
<td>eriodytol</td>
<td>γ-oryzanol</td>
<td></td>
</tr>
<tr>
<td>Sinopic acid</td>
<td>hernmetins</td>
<td>β-sitosterol ferulate</td>
<td>tocopherols</td>
</tr>
<tr>
<td>Syringic acid</td>
<td>hesperetin</td>
<td></td>
<td>tocotrienol</td>
</tr>
<tr>
<td>Vanillyc acid</td>
<td>isorhamnetins, luteolin, peanidin glucoside, tricin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1
For this purpose total lipid of this products are extracted and sterol profiles of these extracts were analyzed by gas chromatography. Rice bran is reported as the most lipid-rich material, with 22.2% total lipid extracted on a dry weight basis. In terms of raw material, the highest phytosterol content is also obtained from rice bran with 4.5 mg/g bran. Sitosterol, campesterol and stigmasterol are determined as major phytosterols in samples however different from these components, it is indicated that rice bran oil contains very high concentrations of cycloartenol and 24-methylene cycloartenol.

In a study that determined the antioxidant activity of different variety of two bran, three different extraction solvent (methanol, ethanol and ethyl acetate) were used shows that antioxidant phyto compounds from rice bran with methanol produces a significantly greater yield than ethanol and ethyl acetate. Presence of tocotrienols or synergistic effect of tocopherols and tocotrienols are reported as a reason of the strong antioxidant activity (Arab et al., 2011).

Rice bran contains 12–22% oil by weight (Sharif et al., 2014). Crude rice bran oil is rich in unsaturated linoleic and oleic fatty acids and bioactive compounds such as γ-oryzanol, phytosterols, tocopherols, and tocotrienols (Friedman, 2014). In addition to nutritious components and health benefits of rice bran oil, some properties such as good stability, appealing flavour and long fry life, provides the rice bran oil in shortening (Liang et al., 2014).

In spite of being an excellent nutrient source, raw rice bran is not suitable for human consumption because of rancidity problem. This problem mainly occurred by lipases and inactivation of the deteriorative enzymes is called stabilization. The employed stabilization approaches are extrusion, micro wave treatment, ohmic heating, dry heat treatment, gamma radiation, parboiling, toasting etc. (Tuncel et al., 2014b).

To evaluate rice bran as potential food ingredients and improve quality and nutrition, several studies were attempted. Especially most of the studies are available which are related to improve flour formulation of bread. Sekhan et al. (1997) prepared leavened pan bread with addition full fat and defatted rice bran flour at 5, 10 and 15%. Reduction in loaf volume and decreasing in overall acceptability of bread are recorded. In similar study, Lima et al. (2002) used full fat and defatted rice bran to evaluated functional behaviour of bread. Results showed that bread hardness, gumminess and chewiness increase depending on level of rice bran. Cohesiveness and springiness were not affected significantly.

In another study, infrared stabilized rice bran is supplemented to white wheat, wheat bran and whole grain breads at levels of 2.5, 5.0 and 10.0 % by Tuncel et al. (2014a). For all types of breads, resilience and cohesiveness showed a significant decreasing trend with the inclusion of stabilized rice bran and a strong correlation was observed between these textural attributes. Crude fat and ash content of all bread types were increased with the inclusion of stabilized rice bran. It is observed that significant and gradual increase in insoluble, and thereby total dietary fiber content of pan breads. The same researchers also evaluated the effects of rice bran addition on the content of B vitamins, minerals and phytic acid content of breads. In all type breads, mineral, phytic acid and vitamin B content is significantly increased especially niacin. It is recorded that the antinutritive content of phytic acid should be taken into account (Tuncel et al., 2014b).

In production of corn flakes and tortilla chips, gelatinized corn flour was supplemented with rice bran from 10 to 30%. It was observed that the maximum breakdown viscosity and color quality was affected and sensory parameters decreased. Percentage of protein increased depending on the level of rice bran (Al-Okbi, 2014). De delahaye et al. (2005) used stabilized rice bran flour in order to enrich wheat flour as a source of dietary fiber, in the preparation of frozen pizza and they also evaluated physical, chemical rheological and sensorial characteristics of the pizzas during the storage period of 2 months at -18 °C. It is observed that increasing the stabilized rice bran content results increase crude fat, ash and dietary fiber content of pizza dough. It is reported that the farinographic curves of pizza dough shows the development time, water absorption and stability decreased, while mixing tolerance index and departure time is not affected by enrichment level.

Conclusion
Phytosterols are defined as plant sterols which have important bioactive properties for human health. Because of lowering cholesterol level in blood, antibacterial, antifungal and antiulcer effects, phytosterols are being used in pharmaceutical and food industry. Rice bran as a by product of milling industry is a good source of phytosterols and also generally used as animal feed. Considering the amount of rice production in the world, the amount of by products will also be high level. In order to adding value to by product and producing healthy food, usage of rice bran is available. Due to nutritional profile, functional characteristics, apparent hipoallergenicity, it has many applications in a diet. In addition to them, using with different bioactive compounds might be able to provide multifunctional foods.

References


EVALUATION OF NUTRITION VALUE OF ROMAN SNAIL’S (*HELIX POMATIA*) MEAT OBTAINED IN LATVIA

Daina Ikauniece1,2, Aleksandrs Jemeljanovs1, Vita Sterna3, Vita Strazdina1

1Laboratory Sigulda, Institute of Food Safety, Animal Health and Environment BIOR, Instituta street 1, Sigulda, Latvia
2 Faculty of Veterinary Medicine, Latvia University of Agriculture, K. Helmaņa street 8, Jelgava, Latvia
3 Department of Agro-ecological Research, State Sende Cereals Breeding Institute, „Dizstende”, Dizstende, Libagi parish, Talsi County, Latvia

Abstract

Roman snail (*Helix pomatia*) meat is a favoured product in many European countries as well as in other continents. Of late, its consumption is growing also in Latvia. Investigations about biochemical composition and nutritional value of snail meat are few. Therefore the following objective was set forth for our research: evaluate the nutrition value of the Roman Snail meat obtained in Latvia. Investigations were performed at the Research Institute of Biotechnology and Veterinary Medicine „Sigra”, of Latvia University of Agriculture from 2011 to 2013. The chemical analyses of 35 samples were done. In the studied samples protein, amino acids, intramuscular fat, fatty acids and cholesterol content were by standard methods determined. The average protein content detected in snail meat samples were 12.86 mg 100 g⁻¹ fat content 1.11 mg 100 g⁻¹. It was calculated the ratio of total saturated and polyunsaturated fatty acids, results are 20.39 and 44.06% of total fatty acids content respectively. Research shows that Snail meat is low in lipids. It could be recommended as meat with excellent nutritional qualities.

*Keywords:* Helix pomatia, nutritional value, biochemical composition.

Introduction

Roman snail (*Helix pomatia*) is one of totally 91 terrestrial snail species found in Latvia. As part of the local fauna Roman Snail has been known since 16th century. The snail meat is mainly consumed as delicacy characterized by a high dietetic value and excellent nutritious traits (Cîrlan, Sindilar, 2009). Research shows that it is rich in protein at the same time being low in lipids (Okonkwo, Anyane, 2009; Ligaszewski et al., 2005; Miletic et al., 1991). Cultivation of Roman snails is rapidly growing in Latvia of late as one of alternative types of non-traditional agricultural production. The society of snail breeders already incorporates 200 snail farms. The breeders associate their market outlet with export of the snail meat. To ensure the competitiveness of locally produced snail meat on European markets, the research needs to be carried out on the quality of the obtained product and ways of improving it.

Objective of the research study: evaluation of the composition of fatty acids in pedal mass of Roman snails found in Latvia in the wild versus that of snails cultivated in a trial farm with an aim of use the results obtained for acquisition of high quality product with excellent organoleptic features.

Materials and Methods

The trial was performed in May-September of 2011 at the Roman Snail Research Facility of the Research Institute of Biotechnology and Veterinary Medicine „Sigra”, of Latvia University of Agriculture LLU. The sampling was performed simultaneously from the wild and from snail farm - snails having received the special supplementary Roman snail feed and snails fed wheat meal and bran. The samples of the pedal mass of Roman snails were drawn three times per season: in spring (May), summer (July) and autumn (September). One aggregate sample consisted of 40-50 snails. After sampling, the snails were refrigerated for 24 hours (+4 °C). Post refrigeration the snails were slaughtered by mechanically breaking the shell and separating the pedal mass and visceral mass. The snail pedal fraction was analysed. The chemical analyses of 35 samples were done. Wild snail meat samples collected in the spring in May. In the studied samples protein, fat, cholesterol content, amino acids and fatty acids composition were determined.

*Crude protein* content was determined as total nitrogen content by Kieldahl method and using coefficient 6.25 for calculation (ISO 937:1974).

*Intramuscular fat* content was made by Sochlet method with hidrolysis procedure (boiling in the hidrocloric acid) using SoxCap 2047 and SOX TEH 2055 equipment (FOSS).

*Amino acids.* Dried, defatted meat samples were hydrolysed with 6N HCl in sealed glass tubes at 100 °C for 23 h. Amino acids were detected using reversed-phase HPLC/MS (Waters Alliance 2695, Waters 3100, column XTerra MS C18 5 μm, 1×100 mm). Mobile phase (90% acetonitrile: 10% deionized water) 0.5 mL min⁻¹, column temperature, 40 °C was used. The identity and quantitative analysis of the amino acids were assessed by comparison with the retention times and peak areas of the standard amino acid mixture.

*Fatty acids.* For the pedal fraction, the percentage of saturated (SFA), mono-unsaturated (MUFA) and polyunsaturated (PUFA) fatty acids were determined. Previously homogenized meat samples were prepared for GLC (gas-liquid chromatography) analysis using direct saponification with KOH/methanol followed by a derivatization with (trimethylsilyl) diazomethane by the method of Aldai et al. (2006).

The statistical processing of data was performed with the data statistical processing software SPSS 17.0 (probability 95% or significance level – p<0.05). For
the evaluation of fatty acid level differences in different snail groups the two sampled populations T-test was used.

**Results and Discussion**

Study established in meat of wild Roman snails and breeding snails (*Helix pomatia*) amount of total protein and total fat (see Fig.1).

![Figure 1. Total protein and total fat content (g 100 g⁻¹) in meat of wild snails and breeding snails](image)

The content of crude protein for wild snails was 12.96±0.95 mg 100 g⁻¹ and while the cultivated snails, which are fed with concentrated feed protein was 13.70±0.66 mg 100 g⁻¹. In Roman snail meat total fat content is very low and can be considered as a dietetic product. The research of Fagbaru et al. (2006) shows that in pedal mass of snails the crude protein content varies from 18.66±0.57% (*Limircolaria* spp.) to 20.56±0.05% (*Archachatina marginata*). The research results of Ligaszewski et al. (2005), on the other hand indicate that crude protein level in pedal mass of wild snail population was higher than that of snails cultivated in breeding facilities for all age groups, while protein level for wild snails at 2–3 years of age was essentially higher (p<0.01). Some authors (Okonkwo, Anyaene, 2009), examining *Helix pomatia* meat content in different regions of Lithuania have established the crude protein level from 11.51±0.03% to 16.60±0.03%. According to data of Zymantiene et al. (2006), the crude protein level in pedal mass of *Helix pomatia* is essentially (p<0.001) lower (14.15±0.76%) than that of pig meat (22.80±0.21). A relatively high protein content was established also in the pedal mass of the snail species *Archachatina*, *Archatina* and *Limircolaria* (Adeyeye, Afolabi, 1996).

At the same time in snails’ meat it was also determined cholesterol level (Fig. 2).

Our study confirms that cholesterol content in meat of wild snail (150.93±11.56) and cultivated snail (174.69±0.21) was relatively high.

Differences in cholesterol levels in wild snails meat and breeding snails meat is not essential (p>0.05). Different results were obtained in studies of Turkish scientists (Ozogul, 2005). Studies with *Archachatina marginata* snail show relatively low cholesterol level.

![Figure 2. Cholesterol content (mg %) in meat of wild snails and breeding snails](image)

The protein composition was determined and results assumed in Table 1.

<table>
<thead>
<tr>
<th>Amino acid symbols</th>
<th>Wild snail (mean±SEM)</th>
<th>Breeding snail (mean±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Val</em></td>
<td>0.441±0.08</td>
<td>0.378±0.02</td>
</tr>
<tr>
<td><em>Leu</em></td>
<td>0.600±0.12</td>
<td>0.496±0.02</td>
</tr>
<tr>
<td><em>Ile</em></td>
<td>0.408±0.08</td>
<td>0.308±0.02</td>
</tr>
<tr>
<td><em>Phe</em></td>
<td>0.429±0.08</td>
<td>0.416±0.03</td>
</tr>
<tr>
<td><em>Lys</em></td>
<td>0.567±0.13</td>
<td>0.409±0.02</td>
</tr>
<tr>
<td><em>Thr</em></td>
<td>0.399±0.06</td>
<td>0.351±0.02</td>
</tr>
<tr>
<td><em>Met</em></td>
<td>0.131±0.02</td>
<td>0.109±0.01</td>
</tr>
<tr>
<td><em>Trp</em></td>
<td>0.417±0.01</td>
<td>0.849±0.01</td>
</tr>
<tr>
<td>Arg</td>
<td>0.465±0.06</td>
<td>1.146±0.63</td>
</tr>
<tr>
<td>Asp</td>
<td>0.748±0.11</td>
<td>0.651±0.03</td>
</tr>
<tr>
<td>Ser</td>
<td>0.365±0.05</td>
<td>0.319±0.02</td>
</tr>
<tr>
<td>Glu</td>
<td>1.110±0.16</td>
<td>1.002±0.07</td>
</tr>
<tr>
<td>Gly</td>
<td>0.541±0.08</td>
<td>0.502±0.04</td>
</tr>
<tr>
<td>Ala</td>
<td>0.559±0.08</td>
<td>0.550±0.03</td>
</tr>
<tr>
<td>Pro</td>
<td>0.426±0.07</td>
<td>0.394±0.02</td>
</tr>
<tr>
<td>Tyr</td>
<td>0.326±0.06</td>
<td>0.288±0.01</td>
</tr>
<tr>
<td>His</td>
<td>0.212±0.04</td>
<td>0.241±0.05</td>
</tr>
<tr>
<td>Hyp</td>
<td>0.451±0.01</td>
<td>0.845±0.01</td>
</tr>
<tr>
<td><em>ΣEAA</em></td>
<td>3.392±0.56</td>
<td>3.316±0.42</td>
</tr>
</tbody>
</table>

*– Essential amino acids (EAA)

The data summarized in Table 1 shows that wild snails meat has more aspartic acid (Asp), serine (Ser), glutamic acid (Glu), glycine (Gly), proline (Pro) and tyrosine (Tyr) than in meat of breeding snail, however the differences between snails groups are not significant (p>0.05). Typically, the hydroxyproline (0.845 g 100 g⁻¹) has more in cultivated snails meat. The content of Leucine detected 0.600 g 100 g⁻¹ in wild snail meat and 0.496 g 100 g⁻¹ in cultivated snails meat. The content of Lysine detected 0.567 g 100 g⁻¹ in wild snail meat and 0.409 g 100 g⁻¹ in cultivated snails meat.

*Helix pomatia* meat is the greatest amount of non essential amino acid glutamic acid – 1.110 g 100 g⁻¹ (wild snail) and 1.002 g 100 g⁻¹ (cultivated snail).
Scientific literature references stating that it was snail meat contains essential amino acids leucine (Leu), isoleucine (Ile), fenilalanine (Phe) and tryptophane (Trp) (Inevbore, Ademson, 1988; Inevbore, 1990; Steievent, 1996; Ebenebe, 2000). Researchers believe that the snail meat contains very high quality protein (Ferhat et al., 2011).

The results of the fatty acid composition in pedal mass of the wild and breeding snails are summarised in Figure 3.

The research data revealed that there are significant differences in levels in the polyunsaturated fatty acids (PUFA) and long-chain poly-unsaturated ω-3 fatty acids (ω-3 PUFA) between wild and breeding snails meet samples (p<0.05).

The content of the poly-unsaturated ω-3 fatty acids (ω-3 PUFA) was slightly higher for the wild Roman snails – 6.03±0.29%, while the amount of ω-6 fatty acids (ω-6 PUFA) is higher for the cultivated snails - 16.36±0.16%. It was found that the meat of cultivated snails contained higher level of PUFA (51.17% on the average), than meat of the wild snails (44.06%). The content of MUFA (12.16±0.24%) and total ω-3 fatty acids (5.66±0.17%) in the meat of cultivated snails is lower, than in meat of the wild snails – MUFA 12.81±0.26% and total ω-3 fatty acids 6.03±0.29% (p>0.05). Content of SFA in the meat of the breeding snails detected 20.84±0.38% higher, than in meat of the wild snails – SFA 20.39±0.81 (p>0.05).

Research data of other scientists on the content of fatty acids both, in the pedal mass of wild Helix pomatia ( Özogul et al., 2005) and Helix aspersa maxima (Çağiltay et al., 2011; Milinsk at al. 2003) are available. In studies of Özogul et al. (2005), for wild Helix pomatia the saturated SFA are dominant (37.87%), while the poly-unsaturated PUFA are a minority (25.83%). In our case, the results are different: Roman snails (Helix pomatia) subjected to a feeding trial, showed higher levels of PUFA (44.06±2.59% and 51.17±1.65% accordingly) than SFA (20.39±0.80% and 20.84±0.38% accordingly). At the same time, the trials of Milinsk (2003) with feeding of plant oils, for snails Helix aspersa maxima have produced data which are very close to those obtained by us. The poly-unsaturated PUFAs are dominant (49.90–57.06%), while SFA content is relatively lower (22.20–26.26%). Different fatty acid contents have been obtained in trials of other authors (Çağiltay et al., 2011) establishing PUFAs at the level of 34.38% and SFAs of 28.76% in pedal mass of Helix aspersa. However data on biochemical indicators of visceral mass of snails are noticeably less available.

Evaluating the levels of separate fatty acids, the highest content by percentage was established for SFAs – palmitic acid (C16:0) and stearic acid (C18:0), MUFA – oleic acid (C18:1 ω-9), and PUFAs – eicosadienoic acid (C20:2 ω-6) and eicosatrienoic acid (C20:3 ω-3) (see Table 2).

The levels of fatty acids C20:2 ω-6 and C20:3 ω-3 were not significant higher in cultivated snail meat, than in wild snail meat. Polyunsaturated fatty acids were found in the highest proportion by percentage, which determines also a higher PUFA amount. We found that the proportion of poly-unsaturated fatty acids for all snails is the highest. Analysing separate fatty acids in pedal mass, we found that the content of α-linolenic acid (C18:3 ω-3) for wild snails was 1.78±0.29%, which is very close to indicators obtained in trials of Özogul (2005) – 1.87±0.01%. Different results however are obtained for snails Helix aspersa. According to Çağiltay (2011) the proportion of α-linolenic acid for these snails is by 3.85% higher while Milinsk et al. (2006) has obtained indicators by 0.74% lower than for wild Helix pomatia in Latvian trial. Our initial research data certify that the fatty acid content found in pedal mass of Latvia’s Helix pomatia is not always similar to fatty acid content established by other authors (Özogul et al., 2005, Çağiltay et al., 2011); similar results have been found in pedal mass of Helix aspersa (Milinsk et al., 2003).

Conclusions

1. Roman snail meat protein content detected 12.96–13.70 g 100 g−1 including essential amino acids 3.1–3.4 mg 100 g−1 and it is evaluated as low in fat 0.78 g 100 g−1 (wild snails) and 1.38 g 100 g−1 (cultivated snails).

2. Snail meat in the greatest amounts of non essential amino acid glutamic acid (1.110 mg 100 g−1 found in wild snail meat and 1.002 mg 100 g−1 in cultivated snail) and least contain methionine (0.131 mg 100 g−1 found in wild snail meat and
0.109 mg 100 g⁻¹ in meat obtained from cultivated snail).
3. The results on the whole, do not contradict to the data found in literature and they certify the richness of meat obtained from Roman snails of Latvia in polyunsaturated fatty acids (44.06–51.17% on the average) and its high biological value.
4. Cholesterol content in meat of wild snail (150.93±11.56 mg%) and cultivated snail (174.69±0.21 mg%) was relatively high.

References
NUTRITIONAL CHARACTERISTICS OF WILD BOAR MEAT HUNTED IN LATVIA

Vita Strazdina¹,², Aleksandrs Jemeljanovs³, Vita Stermu⁴, Daina Ikauniece¹

¹ Laboratory Sigulda, Institute of Food Safety, Animal Health and Environment BIOR, Instituta street 1, Sigulda, Latvia, e-mail: vitastrazdina@inbox.lv
² Faculty of Food Technology, Latvia University of Agriculture, Liela Street 2, Jelgava, Latvia
³ Faculty of Veterinary medicine, Latvia University of Agriculture, K.Helmaņa Street 8, Jelgava, Latvia
⁴ Department of Agro-ecologocal Research, State Stende Cereals Breeding Institute, Dizzemes, Dizstende, Libagi parish, Talši County, Latvia

Abstract

Wild game meat is considered as significant source of healthy food, and its share in consumption in recent years, increasing in size. Investigations about biochemical composition of game meat, including wild boar (Sus scrofa scrofa) meat are not very much. Aim of our investigation was evaluate nutrition value of wild boar meat after hunting in Latvia. Nutritional characteristic of wild boar meat was based on the investigations carried out in different regions of Latvia. In the studied samples protein, amino acids, intramuscular fat, fatty acids, cholesterol and microelement content were determined. The average protein content detected in wild boar meat samples were 20.88 mg 100 g⁻¹ fat content 3.45 mg 100 g⁻¹. It was calculated the ratio of total saturated fatty acids, ω-6 and ω-3, results are 42.98; 13.63 and 3.05% of total fatty acids content respectively. The content of microelements Fe and Zn in samples were 8.25 and 8.52 mg kg⁻¹ it was higher than with meat of domestic animals. The results of investigation confirmed preference of wild boar meat in human health in comparison with beef or pork.

Keywords: game meat, nutritional value, dietetic product.

Introduction

Wild boar is a species that is utilised for food and sport hunting throughout the world and the potential of farming wild boars have stimulated interest in this species as a meat producer. In the last years consumption and assortment of game meat products significantly increased. The public attention is especially paid to inhabitants health and value, through the consumption of wholesome food. Wild game meat characterized by high nutritional value and special sensory properties, desired by consumers is (Soriano et al., 2006; Rywotyczy 2003) considered as significant source of healthy food. Since the amino acid composition of proteins from food animals is similar to human muscle and muscle makes up almost 50% of our body’s weight, meat is an excellent source of the amino acids needed for growth, repair, and maintenance. Similarly to other monogastric animals, meat fatty acid composition of wild boars depends on the diet provided (Di Matteo et al., 2003). This is also evident in depot fat from wild boars where, in contrast to ruminants, double bonds of fatty acids are not hydrogenated during digestion (Meyer et al., 1998). In nature, wild boars eat a great variety of indigenous plants, grains, seeds, roots, fruits, insects, earthworms, slugs and small mammals, with the bulk of food consumed consisting of plant material therefore their meat has balance in vitamins and microelements (Schley, Roper, 2003). The vitamin B₁₂ is important for growth and development of human organism and can only be found in the food of animal origin. Investigations about biochemical composition of game meat, including wild boar (Sus scrofa scrofa) meat are not very much in Latvia.

Aim of our investigation was evaluate nutrition value of wild boar meat after hunting in Latvia.

Materials and Methods

Meat samples of wild boar (Sus scrofa scrofa) muscles (m. logisimus lumborum) were collected in the autumn-winter season. The investigations were conducted at the laboratory of Biochemistry and Microbiology of the Research institute of Biotechnology and Veterinary Medicine „Sigra”. In the studied samples (n=12) protein, fat, ash and cholesterol content, amino acids and fatty acids composition and micronutrient amount were determined. Sample preparation was made in 48 hours after slaughtering or hunting. Meat samples of about 300 g were homogenized with BUCHI B-400 (ISO 3100-1).

Protein content was determined as total nitrogen content by Kieldahl method and using coefficient 6.25 for calculation (ISO 937:1974).

Amino acids. Dried, defatted meat samples are treated with constant boiling 6N hydrochloric acid in an oven at around 110 °C for 23 h. Hydrolyzate diluted with 0.1% formic acid. Sample (2 mL) was filtered using sringe filter with 0.45 μm nylon membran. Amino acids were detected using reversed-phase HPLC/MS (Waters Alliance 2695, Waters 3100, column XTerra MS C18 5 μm, 1×100 mm). Mobile phase (90% acetonitrile: 10% deionized water) 0.5 mL min⁻¹, column temperature 40 °C. Data acquisition was done using program Empower pro.

Intramuscular fat content was made by Sochlet method with hidrolysis procedure (boiling in the hydrochloric acid) using SoxCap 2047 and SOX TEH 2055 equipment (FOSS) (LVS ISO 1443:1973).

Cholesterol content was detected by Blur colorimetric method using spectrometer (Шманик, 1973).

Fatty acids. Homogenized meat samples were prepared for GLC (gas-liquid chromatography) analysis using direct saponification with KOH/methanol followed by
a derivatization with (trimethylsilyl) diazomethane by the method of Aldai et al. (2006) An ACME, model 6100, GLC (Young Lin Instrument Co.) equipped with a flame ionisation detector, and an Alltech AT-FAME analytical column (fused silica 30 m×0.25 mm i.d.) was used. The individual FAMEs (fatty acid methyl esters) were identified according to similar peak retention times using standard mixture Supelco 37 Component FAME Mix. The relative proportions of total saturated fatty acids and unsaturated and ω-6 fatty acids and ω-3 was calculated.

Micronutrient amount of meat are measured according to ISO 6869-2002. Methods are based on comparison of radiation absorption emitted by free metal atoms that are forming by spraying ashed sample and the concentrations of certain metal solutions in the flames. In the laboratory to determine the atomic absorption is used spectrometer Analyst 200.

The experimental design was randomised and data were evaluated by analysis was performed using SPSS 17. One-way ANOVA was used for comparison mean values. Statistical significance was declared at p<0.05. Research provides information on the biochemical composition of wild game meat: protein, fat, cholesterol, fatty acids, amino acids and trace amount that can eat any of us with 100 g of meat.

Results and Discussion

Obtained results of dry matter, protein, intramuscular fat, ash and cholesterol determined in wild boar meat samples assumed in Table 1.

Table 1

<table>
<thead>
<tr>
<th>Biochemical composition of wild boar meat</th>
<th>Wild boar meat</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter, %</td>
<td>25.38</td>
<td>0.62</td>
</tr>
<tr>
<td>Protein, %</td>
<td>20.88</td>
<td>2.99</td>
</tr>
<tr>
<td>Collagen content, %</td>
<td>30.24</td>
<td>1.42</td>
</tr>
<tr>
<td>Connective tissue protein, %</td>
<td>1.45</td>
<td>0.08</td>
</tr>
<tr>
<td>Intramuscular fat, %</td>
<td>3.45</td>
<td>1.67</td>
</tr>
<tr>
<td>Ash, %</td>
<td>1.14</td>
<td>0.13</td>
</tr>
<tr>
<td>Cholesterol, mg 100g⁻¹</td>
<td>98.11</td>
<td>6.27</td>
</tr>
</tbody>
</table>

Content of protein in samples of wild boar meat was determined from 19.55% to 23.18%, average protein content 20.88±2.99%. The results of our investigation are similar with other research findings, where protein content in wild boar meat samples was reported 21.9% (Paleari et al., 2003) or 21.74–22.1% (Postolahe et al., 2011).

Results of investigation showed that the intramuscular fat content of wild boar meat determined from 1.63% to 4.27%, average content 3.45±1.67%. Scientific reports showed a wide range of results, depending of found feed intake the fat content determined: 1.23–4.27% (Quaresma et al., 2011), 3.5–5.2% (Zomborszky et al., 1996) or in the Poland 1.95% (Zmijewska, Korzeniowska, 2001).

The connective tissue protein, which in meat is also decisive for its tenderness, general contains 2.5–12.0% (Honikel, 2009). The connective tissue protein content determined in wild boar meet samples was 1.45%.

The meat samples of wild boar had higher cholesterol content – 98.11 mg 100 g⁻¹ than determined in deer (74.23±2.49 mg 100 g⁻¹) or beef (67.92±7.99 mg 100 g⁻¹) samples (Strazdina, 2012). The cholesterol content in meat from wild boars hunted in Portugal was reported 55.6 mg 100 g⁻¹ for female and 58.7 mg 100 g⁻¹ for male (Quaresma et al., 2011)

The protein composition is more significant for human nutrition as protein content. Not all proteins have the same nutritional value; protein quality strongly depends on its amino acid composition and digestibility.
Amino acid score for SCA in the model protein defined 22, calculated score for wild boar meat is 75. The assumed amino acid score showed that biological value or the ability absorbed protein from wild boar meat to fulfil human amino acid requirements is high. Evaluation of fatty acid composition of wild boar meat was made. Results of determined saturated, monounsaturated and polyunsaturated fatty acids demonstrated in Figure 2.

The results of investigation showed that average content of saturated fatty acids in wild boar meat assumed 42.98% of total fatty acids content. Saturated fatty acids content determined in pigs meat was higher – 45.97% (Jansons, Jemeljanovs, 2013), but not in wild deer meat – 33.34% (Strazdina et al., 2012). Scientific reports show lower saturated fatty acid proportion – 34.72% of total fatty acids in the M. serratus anterior from wild boars shot in the winter in a park in Hungary (Sales, Kotrba, 2013). Scientific reports show higher proportion of MUFA – 38-44% in wild boar meat (Quaresma et. al., 2011, Razmaite et al., 2012) in comparison with our results. The proportion of polyunsaturated fatty acids determined in wild boar meat samples hunted in Latvia – 17% was in agreement with those obtained by Razmaite et al., – 17.9% PUFA determined in smples of male and 18.8% in samples of female (2012). Ratios ω-6 / ω-3 and PUFA / SFA showed in Table 3.

Table 3

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Wild boar meat</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ω-3, % of total fatty acid</td>
<td>3.05</td>
<td>0.21</td>
</tr>
<tr>
<td>ω-6, % of total fatty acid</td>
<td>13.63</td>
<td>0.20</td>
</tr>
<tr>
<td>ω-6/ω-3</td>
<td>6.13</td>
<td>0.31</td>
</tr>
<tr>
<td>PUFA/SFA</td>
<td>0.40</td>
<td>0.05</td>
</tr>
</tbody>
</table>

World Health Organization (2003) recommended ratio PUFA/SFA must be higher than 0.4 and scientific reports showed that domestic animals it has too low 0.1 (Wood et al., 2003). Quaresma reported higher ratio PUFA/SFA – 0.5 (males) to 0.6 (females) and higher ω-6 / ω-3 – 15.5 (females) to 17 (males) in wild boar meat hunted in Hungary because sum of ω-6 were detected 21–24% and sum of ω-3 just 1.4% (Quaresma et al., 2011).

Next to the favorable composition of fatty acids the game meat also contains B vitamins in large quantities. The following vitamins of the meat were examined: A, D, E, B₁, B₃, B₆, B₁₂, Niacin, Pantotenic, Folic acid, results are shown in Table 4.

In M. psoas major from wild boars, 71% of total vitamin E homologues were represented by α-tocopherol (Quaresma et al., 2011). Alpha-tocopherol was higher in adult males (19.2 μg g⁻¹ meat) and females (18.1 μg g⁻¹ meat), compared to youngsters (15.5 μg g⁻¹ meat). Similarly, differences between maturity groups were detected in γ-tocopherol, with mean values of 1.75, 1.61 and 1.14 μg g⁻¹ meat for adult males, adult females and youngsters, respectively. Alpha-tocopherol concentration in wild boar meat reported by Quaresma et al. (2011) was higher than concentrations of γ 15.1–16.3 μg meat found by Jensen et al. (1997).

Red meat contains a number of B vitamins: thiamin (vitamin B₁), riboflavin (vitamin B₂), pantothenic acid, folate, niacin (vitamin B₆), vitamin B₉ and B₁₂. Meat, fish and animal-derived foods, such as milk, are the only foods that naturally provide vitamin B₁₂. For this reason, if you exclude such foods from your diet, you are at risk of having inadequate intakes. Red meat is a rich source of vitamin B₁₂ (Mann, 2000) and about 35% of vitamin B₁₂ intake comes from meat and meat products (Henderson, 2003). Dietary intakes of vitamin B₁₂ are lower from vegetarian diets, and are particularly low in vegan diets (Phillips, 2005; Li et al., 2005) (which contain no animal foods), thus indicating the important contribution of meat and animal - derived products to B₁₂ intake.

The following chemical parameters of the meat were examined: calcium, phosphorus, potassium, sodium, magnesium, iron, manganese, potassium, sodium,
magnesium showed in Table 5. The content of microelements Fe and Zn in samples were determined – 8.25 and 8.52 mg kg⁻¹. The amount of iron, phosphorus and potassium determined in the m. longissimus dorsi was 7.701 mg, 752 mg and 1114 mg per 100 grams of dry matter, respectively.

Table 5

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Wild boar meat</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macronutrients, mg 100 g⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca</td>
<td>3.22</td>
<td>0.21</td>
</tr>
<tr>
<td>P</td>
<td>91.15</td>
<td>0.31</td>
</tr>
<tr>
<td>K</td>
<td>930.71</td>
<td>3.17</td>
</tr>
<tr>
<td>Na</td>
<td>20.43</td>
<td>0.74</td>
</tr>
<tr>
<td>Mg</td>
<td>7.31</td>
<td>0.33</td>
</tr>
<tr>
<td>Fe</td>
<td>8.25</td>
<td>0.63</td>
</tr>
<tr>
<td>Trace elements, mg 100 g⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>8.52</td>
<td>0.45</td>
</tr>
<tr>
<td>Cu</td>
<td>0.04</td>
<td>0.00</td>
</tr>
<tr>
<td>Mn</td>
<td>0.03</td>
<td>0.00</td>
</tr>
</tbody>
</table>

According to Świergosz, Perzanowski, Makosz, and Bilek (1993), means for copper determine 6.4–7.4 mg kg⁻¹ dry matter, iron 99.2–110.4 mg kg⁻¹ dry matter and manganese 2.9–3.4 mg kg⁻¹ dry matter did not vary to a large extent between two different regions in the south eastern part of Poland (Sales, Kotrba, 2013).

Conclusions

Result collected in the study revealed, that meat from wild boars, in many aspects, was characterised as higher quality in comparison to meat from domestic pigs. The percentage of proteins in meat is content 20.88±2.99%, and the proteins also have an above-average biological value- sum of Indispensable amino acids detected 7.30 g 100 g⁻¹. The most important vitamins are B₁ and B₂, as well as vitamin B₁₂ that can be found only in food of animal origin. Nutritional value of game meat is completed with micro elements of vital essence: content of Fe, Zn was determined – 3.44 and 3.73 mg kg⁻¹ respectively.

Acknowledgment

Publication and dissemination of research results has been made due to the funding of the ERAF Project „Promotion of scientific activities of LUA”, Contract Nr. 2010/0198/2DP/2.1.1.2.0/10/APIA/VIAA/020.

References


DRIED VENISON PHYSICAL AND MICROBIOLOGICAL PARAMETERS CHANGES DURING STORAGE

Ilze Gramatina, Laima Silina, Liga Skudra, Tatjana Rakcejeva

Department of Food Technology, Faculty of Food Technology, Latvia University of Agriculture, Liela Street 2, Jelgava, Latvia, e-mail: ilzegramatina@inbox.lv

Abstract

Venison due to the lower fat content in muscles has gained increased popularity in recent years comparing to pork or beef. The aim of the current research was to determine changes in physical and microbiological parameters of dried venison during storage. For the experiments the meat (0.02±4.00±7.00 cm) pieces were marinated in “teriyaki sauce” marinade (composition: teriyaki sauce, sweet and sour sauce, taco sauce, soy sauce, American BBQ sauce hickory, sesame oil, garlic, garlic salt, tabasco red pepper sauce) at 4±2 °C temperature for 48±1h. To improve the meat textural properties sodium monophosphate (E339) was added in part of marinade too. After marinating meat samples were dried in microwave-vacuum drier MUSSON–1, packaged in vacuum pouches made from polymer film (PA/PE) with barrier properties and storage for 4 months at +18±1 °C temperature in dark place. The following physical and microbiological parameters of dried meat were evaluated after 35, 91 and 112 storage days using standard methods: pH (AACC 02-52), colour by Colour Tec PCM/PSM (CIE L*a*b* system), texture by TA. XT. plus Texture Analyser, the total count of mesophilic aerobic and facultative anaerobic microorganisms (Ref. Nr. 01–140), lactic acid bacteria (Ref. Nr. 01–135). Experimentally it was ascertained, that the shelf-life of dried venison with sodium monophosphate additive is 91 day and without sodium monophosphate additive – 112 days. Significant differences were detected in meat color and texture changes. However, dried meat color stability and texture is possible to provide during storage by adding of sodium monophosphate at marinade.

Keywords: venison, drying, microwave-vacuum drier.

Introduction

Venison due to the lower fat content in muscles has gained increased popularity in recent years. It is lower in calories, fat and cholesterol content comparing to lamb, pork or beef. Colour, tenderness and juiciness are the most important parameters for consumers in meat (Vergara et al., 2003). Currently, marination is widely used by consumers and producers to improve meat tenderness and juiciness (Ergezer, Gocke, 2011).

Marination is the process of meat soaking or injecting a solution containing ingredients such as vinegar, lemon juice, wine, soy sauce, brine, essential oils, salts, tenderizers, herbs, spices and organic acids to flavour and tenderize meat products (Kargiotou et al., 2011; Pathania et al., 2010). The functionality of most marinades directly depends on their ingredients. The most common and important ingredients of acidic marinades are organic acid solutions (acetic acid, lactic acid, citric acid, etc.), vinegars, wine, or fruit juices (Burke, Monahan, 2003).

Drying is a method of food preservation that works by removing water from the food, which inhibits the growth of bacteria and has been practiced worldwide since ancient times to preserve food. A solar or electric food dehydrator can greatly speed the drying process and ensure more consistent results. Water is usually removed by evaporation (air drying, sun drying, smoking or wind drying). Bacteria, yeasts and moulds need the water in the food to grow, and drying effectively prevents them from surviving in the food (Bowser et al., 2009).

Hot-air drying has been to date the most common drying method employed for food materials. However, this method has many disadvantages, including poor quality of dried products, low energy efficiency and a long drying time. Microwave-vacuum drying is a novel alternative method of drying, allowing to obtain products of acceptable quality. It permits a shorter drying time and a substantial improvement in the quality of dried materials, in relation to those dried with hot air and microwaves drying method (Bondaruk et al., 2007).

Microwaves and radiofrequency waves are electromagnetic waves that are given in concrete frequency bands and both are enclosed into the dielectric heating (Knoerzer et al., 2006). Microwave energy is adequately combined with vacuum technology; it enables to keep temperatures within a low range (Yongswatdigul, Gunasekaran, 1996). If a suitable control system is used, microwaves provide high quality products with less nutrient loss, more flavour retention, less colour change, a natural appearance (Drouzas, Schubert, 1996; Erle, Shubert, 2001).

The aim of the current research was to determine changes in physical and microbiological parameters of dried venison during storage.

Materials and Methods

The experiments were carried out at the Department of Food Technology, Latvia University of Agriculture, in Year 2013.

The object of the research

The meat of farmed red deer (Cervus elaphus) was obtained from a local farm Saulstari 1, located in Sigulda region, Latvia.

Components of marinade

Teriyaki sauce marinade (composition: teriyaki sauce, sweet and sour sauce, taco sauce, soy sauce, American BBQ sauce hickory, sesame oil, garlic, garlic salt, tabasco red pepper sauce) was used for venison marinating.
Meat marinating

Marinating process of the meat included the following steps:

- *Musculus longissimus lumborum* muscle from venison was manually divided into smaller pieces of the size 0.02×4.00×7.00 cm, and teriyaki sauce marinade was added. To improve the meat textural properties sodium monophosphate was added in 1.2% from total amount of marinade according to Ergezer and Gocke (2011).
- Prepared samples were marinated at 4±2 °C temperature for 48±1 h.

Meat drying and packaging

After marinating, the microwave-vacuum drier Musson-1 (OOO Ingriedient, Russia) (Vacuum microwave drier MUSSON-1, 2007) was used for venison drying.

Dry meat samples were packaged in the vacuum pouches made from polymer film (PA/PE) with barrier properties.

Dried meat storage conditions

Packaged dry meat samples were stored at dark place at 18±1 °C temperature for 112 days. Quality parameters of dry venison samples were analysed after 0, 35, 91 and 112 days of storage.

Standard methods for samples quality analysis

Microbiological parameters were analysed:

- the total plate count (TPC) of mesophilic aerobic and facultative anaerobic microorganisms according to the standard method LVS EN ISO 4833:2003 (Ref. Nr. 01–140);
- lactic acid bacteria according to the standard method ISO 9332:2003 (Ref. Nr. 01–135).

The pH was measured using Jenway 3520 pH Meter (Jenway, AK). The pH electrode was dipped into a mixture of homogenized sample and distilled water (1:10), according to AACC02-52. For pH analyses, meats samples were homogenised using a household blender according to ISO 17604:2003 standard procedure.

Colour changes were determined with the ColorTec PCM/PSM – colorimeter (Accuracy Microsensors Inc., USA) – CIE L* a* b* system. Colour values were recorded as L* (brightness), a* (-a, greenness, +a, redness) and b* (-b, blueness, +b, yellowness).

Meat tenderness was evaluated by shear force using a TA. XT. plus Texture Analyser (Stabel Micro Systems, UK) equipped with a Warner-Bratzler blade.

Mathematical data processing

Microsoft Excel software was used for the calculation of mean arithmetical values and standard deviations. SPSS 20.0 software was used to determine the significance of research results and ANOVA analyses to explore the impact of factors and their interaction, the significance effect (p-value).

Results and Discussion

Technological parameters

During experiments optimal technological parameters for marinated meat (2.5±0.1 kg) drying in microwave-vacuum drier were developed: temperature – +36±2 °C and drying time – 80 min, one working cycle, pressure 7.5/9.3 kPa, container rotation speed – 6 rpm, the total energy input 2856 kJ kg⁻¹ initial load.

Meat was dry until content of moisture in samples without and with sodium monophosphate accordingly achieved 25.08±0.66% and 34.78±0.60% corresponding to Yang et al. (2009).

Microbiological parameters

The total plate count (TPC) of mesophilic aerobic and facultative anaerobic microorganisms (CFU g⁻¹) in dry meat not regulates with European Commission regulation No. 2073/2005. Therefore for obtained data interpretation Russian sanitary and epidemiological regulations Enactment date of sanitary and epidemiological rules and regulations “Hygienic Requirements for Safety and Nutrition Value of Food Products / Sanitary Rules and Regulations (SanPin) 2.3.2.1078–01” with permissible limit as 1×10⁴ CFU g⁻¹ (Санитарно–эпидемиологические правила..., 2002) was used. Experimentally was detected that before drying TPC CFU g⁻¹ of marinated meat without sodium monophosphate was 2.32 log CFU g⁻¹ and with sodium monophosphate – 2.46 log CFU g⁻¹, obtained results was not exceed permissible level.

Figure 1. TPC dynamics of dried venison during storage

There are not found significant (p=0.117) changes of TPC of meat samples after drying.

Significant TPC changes of dry venison samples (Figure 1) with sodium monophosphate were indicated after 91 days storage, as a result the volume of TPC reach permissible level – 1×10⁴ CFU g⁻¹, and it was 3.99 log CFU g⁻¹. Such changes could be explained by relatively higher moisture content in analysed meat samples. However in dry venison samples without sodium monophosphate TPC CFU g⁻¹ reach permissible level after 112 storage days and it was – 3.79 log CFU g⁻¹. Therefore the recommendable dry venison with sodium monophosphate shelf-life

38
could be 91 days, but of venison without sodium monophosphate – 112 days.
The count of lactic bacteria (Fig. 2) in marinated venison without sodium monophosphate was 1.99 log CFU g\(^{-1}\), however in samples with sodium monophosphate – 1.90 log CFU g\(^{-1}\). After drying a count of lactic acid bacteria increases as follow in meat samples without sodium monophosphate until 2.00 log CFU g\(^{-1}\), with sodium monophosphate – 1.98 log CFU g\(^{-1}\) (p<0.05).

The changes of count of lactic acid bacteria very tightly connected with meat samples pH value as a result a pH changes of count of lactic acid bacteria mainly depends on optimal pH value. Myoglobin is found in fresh meat, but after some hours of store at air ambiance or after of heat treatment process meat colour turn of brown or grey-brown, due to metmyoglobin formation. Meat colour stabilization is possible to prevent development of metmyoglobin.

**Colour**

The changes of colour parameters \(L^*\), \(a^*\) and \(b^*\) values of dry venison during storage are shown in Table 1. Colour parameter \(L^*\) value of marinated meat with sodium monophosphate was higher comparing with marinated meat without sodium monophosphate; differences was not significant (p>0.05). \(L^*\) value of marinated venison without sodium monophosphate was 30.66±1.60, but with sodium monophosphate – 31.37±2.05. However, colour of meat stabilizes optimal pH value. Myoglobin is found in fresh meat, but after some hours of store at air ambiance or after of heat treatment process meat colour turn of brown or grey-brown, due to metmyoglobin formation. Meat colour stabilization is possible to prevent development of metmyoglobin.

There are not found significant differences in count of lactic acid bacteria between analysed dry venison samples during storage (p=0.166). From other side the higher count of lactic acid bacteria was established in dry meat with sodium monophosphate additive. Obtained results could be explained with higher moisture content in analysed dry meat samples with sodium monophosphate, that create favourable conditions for development of lactic acid bacteria. The changes of count of lactic acid bacteria very tightly connected with meat samples pH value as a result a pH value was less and the amount of lactic acid bacteria increases (Skandamis, Nychas, 2002).

**Physical parameters**

\(\text{pH value}\) of marinated venison without sodium monophosphate before drying was 5.14±0.06, but with sodium monophosphate – 5.32±0.04.

![Figure 2. Lactic acid bacteria dynamics in dried venison during storage](image.png)

**Figure 2. Lactic acid bacteria dynamics in dried venison during storage**

In scientific literature is mentioned that in result of using of phosphate pH value of meat increase (Sheard, Tali, 2004; Murphy, Zerby, 2004).

**Effects of storage time on the colour values**

\[(\text{L}^*, \text{a}^*, \text{b}^*)\] of dry venison

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Storage days</th>
<th>Dry venison meat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>(L^*)</td>
<td>0</td>
<td>31.05±2.21</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>35.11±2.62</td>
</tr>
<tr>
<td></td>
<td>91</td>
<td>36.89±2.81</td>
</tr>
<tr>
<td></td>
<td>112</td>
<td>–</td>
</tr>
<tr>
<td>(a^*)</td>
<td>0</td>
<td>8.45±9.68</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>7.02±8.76</td>
</tr>
<tr>
<td></td>
<td>91</td>
<td>6.16±7.65</td>
</tr>
<tr>
<td></td>
<td>112</td>
<td>–</td>
</tr>
<tr>
<td>(b^*)</td>
<td>0</td>
<td>5.03±4.64</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>4.03±5.57</td>
</tr>
<tr>
<td></td>
<td>91</td>
<td>3.98±1.19</td>
</tr>
<tr>
<td></td>
<td>112</td>
<td>–</td>
</tr>
</tbody>
</table>

A – with sodium monophosphate; B – without sodium monophosphate.

After drying colour parameter \(L^*\) value of meat decrease and meat samples turns darker, but obtained changes was not significant (p>0.05). The significant differences was established (p<0.05) in \(L^*\) value between dry meat samples without/sodium monophosphate in long period of storage time. Therefore, the experimental date indicates, that sodium monophosphate stabilizes colour of dry meat samples during storage.

Colour parameter \(a^*\) value of marinated meat without sodium monophosphate (5.03±2.67) was lower comparing with marinated meat with sodium monophosphate (6.93±2.71), but detected differences was not significant (p>0.05).
Colour parameter a* values of meat samples without/with sodium monophosphate increased significantly (p=0.024; p=0.047) after drying, and it was 7.99±1.51 and 8.45±9.68 respectively. Such colour changes could be explained with moisture content decreases in meat samples after drying. As a result a colour of meat becomes more dark and red. Dry venison without sodium monophosphate color parameter a* value after 112 day of storage increase till 5.55±8.94 (significantly p=0.001), but of dry venison with sodium monophosphate a* colour parameter value after 91 day increase till 6.16±7.65, what is significant (p=0.0001). The O’Sullivan et al. (2002) indicated, that the colour parameter a* value usually was tightly connected with content of oxymyoglobin in meat. Therefore it is possible to conclude, that after heat treatment due to metmyoglobin formation colour parameter a* value decreases in meat. As a result meat colour turn of brown or grey-brown. 

Colour parameter b* value of marinated venison without sodium monophosphate was obtained as 15.77±5.96, with sodium mono phosphate – 14.79±7.10 (p=0.05). After drying of marinated venison samples without/with sodium monophosphate colour parameter b* value decrease significantly (p=0.006; p=0.021) respectively, but differences between samples was not significant (p>0.05). b* colour parameter value of dry venison without sodium monophosphate was 6.19±7.30, with sodium monophosphate – 5.03±4.64. b* colour parameter value decrease significantly (p=0.0001) in dry venison samples without sodium monophosphate and in samples with sodium monophosphate (p=0.009) during storage (Table 1). After mathematical data processing significant differences was established between analysed samples without/with sodium monophosphate additive (p=0.05) during storage. It is necessary to indicate, according to the Franco et al., (2012) in scientific literature was not found explanation about b* colour parameter value changes in meat during storage.

**Tenderness (shear force)**

Experimentally it was detected that marinated meat with sodium monophosphate additive was softer than marinated meat without sodium monophosphate (p=0.05). Results indicated that marinated venison without sodium monophosphate tenderness was 23.42±6.72 N, and with sodium mono phosphate – 16.20±1.13 N or 1.45 times less.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Storage days</th>
<th>Dry venison meat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shear force, N</td>
<td>0</td>
<td>95.3±9.46</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>112.3±2.27</td>
</tr>
<tr>
<td></td>
<td>91</td>
<td>151.6±4.54</td>
</tr>
<tr>
<td></td>
<td>112</td>
<td>–</td>
</tr>
</tbody>
</table>

A – with sodium monophosphate; B – without sodium monophosphate.

After drying tenderness of meat samples increase significantly (p=0.05), because a content of moisture increase and finally structure mechanical properties of dried meat changed. Tenderness of dry venison without sodium monophosphate was 120.77±7.71 N, whereas with sodium monophosphate – 95.33±9.46 N (Table 2). During research it was proved that tenderness of meat samples with/without sodium monophosphate additive significantly increase (p<0.05) during storage. However dry meat samples with sodium monophosphate additive was softer (p>0.05), because it’s higher pH value.

**Conclusions**

Experimentally it was ascertained, that the shelf-life of dried venison, packaged in vacuum pouches made from polymer film (PA/PE) with barrier properties and stored at a temperature +18±1 °C in the dark place, with sodium monophosphate additive could be 91 day and without sodium monophosphate additive – 112 days. There are not found significant differences in pH values of analyses dried meat sample during storage comparing with the parameters of just dried meat. Significant differences were detected in meat color and tenderness changes during meat storage. Dried meat color stability and structure mechanical properties possible provide by adding of sodium monophosphate at marinade.

**Acknowledgment**

The work of the doctoral student L. Silina is supported by the ESF project No.2009/0180/1DP/1.1.2.1.2./09/IP1A/V1AA/017. This research and publication have been prepared also within the State Research Programme ‘Sustainable use of local resources (earth, food, and transport) – new products and technologies (NatRes)’ (2010–2013). Project No. 3. ‘Sustainable use of local agricultural resources for development of high nutritive value food products (Food)’.

**References**


19. Санитарно-эпидемиологические правила и нормативы “Гигиенические требования к безопасности и пищевой ценности пищевых продуктов, СанПин 2.3.2.1078–01” (01.07.2002) / Enactment date of sanitary and epidemiological rules and regulations “Hygienic Requirements for Safety and Nutrition Value of Food Products. Sanitary Rules and Regulations (SanPin) 2.3.2.1078–01” (01.07.2002).
FOODBALT 2014

THE DIFFERENT PROTEIN SOURCES FEEDING IMPACT ON THE QUALITY OF PORK

Lilija Degola
Institute of Agrobiotechnology, Faculty of Agriculture, Latvia University of Agriculture, Liela street 2, Jelgava, Latvia, e-mail:lilija.degola@llu.lv

Abstract

The study was conducted to determine the quality of pork after feeding soybean meal and peas as protein sources to fattening pigs. The research was done in two pig farms in Latvia and four fattening pig groups were conducted (two in each holding). In holdings one pig group was soybean meal and the other peas group with 10 animals each. The pig groups received different amount of the protein feeds (soybean meal 15%, peas 15% and 28%) blended into a mixture of concentrated feed. The results showed that 15 and 28% inclusion of pea in finisher pig diets about 3–5% increase LWG. The higher pig ADG resulted on the increasing of fat in the body at the similar FC per 1 kg LWG. The IF about 0.68 and 0.48 kg were more in peas pig groups with significant differences (p<0.001) between soybean pig groups. For pigs, which fed peas, average backfat were of 14.3 mm, 2 and 1.3 mm more than the one of pigs in soybean groups. There were no differences of ash content and pH in Longissimus dorsi muscle, but moisture, fat, CP and cholesterol content were significantly different (p<0.05; p<0.01; p<0.001) between soybean and peas groups. Fat content in Longissimus dorsi muscle was 2.5 till 2.8% higher (p<0.01) for peas group pigs. The fatty acid content were not high, but were significantly (p<0.001, p<0.01, p<0.05) different with pigs of soybean groups. The highest values of nonessential amino acids were measured in glutamic (3.09–3.54 g). The lowest value were found out in tryptophane (0.245 g) and proline (0.37 g) in soybean group, but in peas groups the content of proline were (0.772–0.779 g) and was more twice times higher than tryptophan.

Keywords: pig, peas, nutrition, amino acid, pork.

Introduction

The main feed for pigs are cereal grains which commonly used as feed in industrial pig production. The cereal grains contain insufficient quantities of several of the indispensable amino acids such as lysine, threonine and sulfur containing amino acids (Sauer et al., 1977). Therefore, it is essential that the pigs’ diet contain a supplementary source of these limiting amino acids. Soybean meal is often used as the main supplement of amino acids in pig feed (Jezierny et al., 2010). In temperate environments, soybeans are difficult to cultivate and the pig industry relies heavily on imported soybean meal. However, as the transportation cost for feed increases, pig producers will have to maximize the use of locally produced feedstuffs. Therefore, it is important that alternative sources of supplementary protein be developed. In temperate environments, the one crop which is potential for use in pig diets is the peas. Field peas (Pisum sativum L.) have been grown for centuries in many parts of the world. Historically, field peas have been produced mainly for human consumption, but during the last years, the industry has also found markets for field peas in pig feeding. Because the goal of this study was to evaluate the effect of including different levels of pea in pig diets on performance of finisher pigs, carcass and meat quality.

Materials and Methods

Animals and housing. The research was done in two pig farms in Latvia. The four fattening pig groups were conducted (two in each holding), according of pig origin, age and liveweight. In holdings one pig group was (1 and 2) soybean meal and the other (1 and 2) peas group, with 10 animals each. The selected pigs were crossbreed – Large White×Landrace in both farms. Finisher pigs were housed on concrete floors with shavings and access to drinking water at all times. Each pig was allowed access to its individual feeder for 30 min twice daily. The trial was run for 50–54 days (FD) and concluded when the pigs reached an average weight of 94–113 kg.

Diets and Performance Measures. Commercial sources of pea (variety ‘Respect’) and soybean meal were obtained for the experiment. The soybean meal groups in both holdings received soybean meal 15% as alone protein feed in diet, but peas’ groups pigs received 28% peas in first holding and 15% peas in second holding, for check out the peas amount influencing to pork quality. The protein feeds were blended into a mixture of concentrated feed. The other ingredients in diets were kept constant for all groups and included barley, wheat, triticale and trace element-vitamin premix. The pig feed mixture was prepared without crystalline amino acids. The diets were formulated to be isoenergetic for metabolize energy (ME), with the same crude protein (CP), Lysine (Lys) content (calculated for Feed Catalog), and to meet the minimum requirements of Ca and P by modifying the inclusion macroelements and vitamins (Table 1). Analysis of feed samples for dry matter, crude protein, crude fiber, fat, ash, Ca and P were determined in scientific laboratory of agronomik analysis of Latvia using such methods: for dry matter (DM) – forage analyses, USA, met. 2.2.1.1:1993, crude protein (CP) LVS EN ISO 5983-2:2009, crude fiber (CF) ISO5498:1981, fat ISO6492:1989, ash ISO 5984:2002/Cor1:2005, Ca LVS EN ISO 6869:2002, P ISO 6491:1998. Individual body weight, feed consumption (FC) was recorded on a weekly basis. Slaughter and carcass Quality Measurements. Pigs were slugged at a commercial slaughterhouse via
Pig fattening results showed that pigs growth intensity was high in all study groups of pigs (Table 2). The average daily liveweight gain (ADG) for the pigs were from 850±7.48 till 915±10.4 g, slightly higher in peas groups. Although soybean meal protein digestibility is higher (87%) than pea protein (only 79%) (Jezierny et al., 2011), but about 5% higher and significantly different (p<0.05) live weight gain (LWG) showed the pig group in the second holding, which fed in the feed 15% peas, and in the first holding pig daily weight gain (28% peas in feed) was 3% higher compared to the soybean group (Table 2).

<table>
<thead>
<tr>
<th>Pig fattening results (n=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Start LW, kg</td>
</tr>
<tr>
<td>End LW, kg</td>
</tr>
<tr>
<td>LWG, kg</td>
</tr>
<tr>
<td>% LWG</td>
</tr>
<tr>
<td>DF</td>
</tr>
<tr>
<td>ADG, g</td>
</tr>
<tr>
<td>FC per 1 kg LWG, kg</td>
</tr>
</tbody>
</table>

Values are presented as mean ±standard error. a,b p<0.05

For pigs the most important amino acid is lysine, which is necessary for building muscle tissue (Guoyao Wu et al., 2013). The digestibility of most amino acids in field peas is similar to that in soybean meal, but pea protein has relatively low concentration of methionine, cysteine and tryptophan. It is necessary to pay attention to the concentrations of these amino acids and often need to include crystalline sources. In our trial was not determined these amino acids in the pig diets because the higher pig daily gain may resulted on the increasing of fat in the body at the similar feed consumption per 1 kg live weight gain. Results obtained for pigs confirm results from previous research demonstrating that there are no negative effects of including 36% field peas in diets fed to finishing pigs (Stein et al., 2004). Inclusion of 30% field peas is also was reported not to compromise pig performance. The researchers results showed that even higher inclusion rates of peas may be used (66%) and that peas can substitute all the soybean meal in diets without negatively affecting performance and feed intake (Stein et al., 2006), in addition formulated of the digestible indispensable amino acids in pig diets is obligatory. The other research showed that including pea or faba bean in finisher pigs’ diets

Results and Discussion
Concentrated feed mixtures of chemical analyzes showed that the pigs received a full and balanced feed (Table 1). Based on fattening pig nutrition standards (NRC, 2012) the necessary crude protein is 14–16% with the lysine content 0.5–0.8% of the dry matter, depending on the liveweight (LW).

The chemical content of feed in dry matter

<table>
<thead>
<tr>
<th>Indices</th>
<th>First holding</th>
<th>Second holding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 soybean group</td>
<td>1 peas group</td>
</tr>
<tr>
<td>DM, %</td>
<td>88.89</td>
<td>88.00</td>
</tr>
<tr>
<td>ME MJ, kg</td>
<td>13.30</td>
<td>13.40</td>
</tr>
<tr>
<td>CP, %</td>
<td>15.27</td>
<td>14.62</td>
</tr>
<tr>
<td>Lys, %</td>
<td>0.77</td>
<td>0.73</td>
</tr>
<tr>
<td>CF, %</td>
<td>4.36</td>
<td>4.67</td>
</tr>
<tr>
<td>Fat, %</td>
<td>1.74</td>
<td>1.67</td>
</tr>
<tr>
<td>Ash, %</td>
<td>5.57</td>
<td>4.89</td>
</tr>
<tr>
<td>Ca, %</td>
<td>0.90</td>
<td>0.79</td>
</tr>
<tr>
<td>P, %</td>
<td>0.59</td>
<td>0.56</td>
</tr>
</tbody>
</table>

Electrical stunning, followed by exsanguinations, and carcases were dehaired via scalding, eviscerated, and split vertically down the midline. Hot carcases weights were obtained and backfat depth was measured at a specific site [i.e., the head of the last rib, 6 cm from the mid back line, using a probe (Introscope Optimal Probe)]. The length of carcase (CL) was measured in a straight line from the forward edge of the first rib to the forward edge of the twitch bone and muscle-eye (LM) area with the planimeter. The internal fat (IF) was removed from carcases and weighed, the loin muscle (LMW) also was removed without fat from the left side of each group 3 pig carcases and weighed. Left side of carcase was divided into fractions for determination ham (HW), bone (BW) and meat weight (MW). The samples of meat were taken from the musculus longissimus lumborum et thoracis 24 hours post mortem and subsequently subjected to the chemical analysis. Chemical content of pork analyzed for such indices: fat content (LVS ISO 1443:1973), moisture (LVS ISO 1442:1997), crude protein content (CP) (LVS ISO 937:1978), ash (ISO 936:1998), pH (LVS ISO 2917:2004), cholesterol (BIOR-T-012-132-2011), unsaturated fatty acids–αlfa – linolenic (C18:3), arachidonic (C20:4), eicosapentaenoic (C20:5), linoleic (C18:2), oleic (C18:1) and palmitoleic (C16:1) (BIOR-T-012-131-2011). Amino acid composition of the muscle was analyzed with methods ISO13903:2005, but tryptophan content by method EN ISO 13904, HPLC. The content of each individual amino acid was calculated on g 100 g of wet matter basis.

Statistical Analysis. The data were processed with MS Excel mathematical program. The results were compared using t-test. t-test was carried out on the data for growth performance and meat quality.

<table>
<thead>
<tr>
<th>Results</th>
<th>First holding</th>
<th>Second holding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 soybean group</td>
<td>1 peas group</td>
</tr>
<tr>
<td>Start LW, kg</td>
<td>46.9±0.43</td>
<td>46.3±0.42</td>
</tr>
<tr>
<td>End LW, kg</td>
<td>94.5±0.36</td>
<td>95.7±0.67</td>
</tr>
<tr>
<td>LWG, kg</td>
<td>47.9±0.77</td>
<td>49.4±0.95</td>
</tr>
<tr>
<td>% LWG</td>
<td>100</td>
<td>103</td>
</tr>
<tr>
<td>DF</td>
<td>54</td>
<td>54</td>
</tr>
<tr>
<td>ADG, g</td>
<td>887±21.8</td>
<td>915±10.4</td>
</tr>
<tr>
<td>FC per 1 kg LWG, kg</td>
<td>2.88</td>
<td>2.98</td>
</tr>
</tbody>
</table>

Values are presented as mean ±standard error. a,b p<0.05
from 7.5–30% slightly reduced finisher daily gain, as tended to reduce over initial levels only (Smith et al., 2013). Carcass indicators show a tendency to accumulate more fat tissue in pigs, which fed pea in the feed. The internal fat about 0.68 and 0.48 kg were more in pea groups with significant differences (p<0.001) between soybean pig groups (Table 3). The significant differences was found also of meat weight in both holdings (p<0.05 and p<0.001), of muscle-eye area in first holding and of ham weight in second holding (p<0.05). The pigs which fed peas had a little smaller loin muscle areas (LM).

### Table 3

**Carcass traits (n=12)**

<table>
<thead>
<tr>
<th>Indices</th>
<th>First holding</th>
<th></th>
<th>Second holding</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 soybean group</td>
<td>1 peas group</td>
<td>2 soybean group</td>
<td>2 peas group</td>
</tr>
<tr>
<td>CW, kg</td>
<td>72.2±0.35</td>
<td>82.3±0.52</td>
<td>89.8±0.61</td>
<td>90.1±0.69</td>
</tr>
<tr>
<td>CL, cm</td>
<td>107±0.58</td>
<td>108.7±0.88</td>
<td>109±0.58</td>
<td>108.3±0.33</td>
</tr>
<tr>
<td>FT, mm</td>
<td>12.3±0.88</td>
<td>14.3±1.2</td>
<td>13±0.57</td>
<td>14.3±0.33</td>
</tr>
<tr>
<td>LM, cm²</td>
<td>52.5±0.68</td>
<td>50.5±0.21</td>
<td>51.3±0.16</td>
<td>50.3±0.88</td>
</tr>
<tr>
<td>HW, kg</td>
<td>10.6±0.19</td>
<td>10.7±0.06</td>
<td>11.5±0.29</td>
<td>12.5±0.19</td>
</tr>
<tr>
<td>IF, kg</td>
<td>1.32±0.01</td>
<td>2±0.05</td>
<td>1.85±0.03</td>
<td>2.33±0.04</td>
</tr>
<tr>
<td>LMW, kg</td>
<td>2.88±0.04</td>
<td>2.89±0.09</td>
<td>3.22±0.02</td>
<td>3.23±0.04</td>
</tr>
<tr>
<td>BW, kg</td>
<td>9.9±0.06</td>
<td>13±0.05</td>
<td>13.4±0.12</td>
<td>10.8±0.15</td>
</tr>
<tr>
<td>MW, kg</td>
<td>62.3±0.23</td>
<td>69.3±0.58</td>
<td>76.4±1.21</td>
<td>79.2±0.41</td>
</tr>
</tbody>
</table>

Values are presented as mean ±standard error. a,b,c,d,f,p<0.05; c,d,e p<0.01

For pigs, which fed peas, average backfat were of 14.3 mm, which is 2 and 1.3 mm more than the of soybean groups pigs. The results of the carcass traits of this experiment confirm previous studies. It has been reported from European studies that the lean meat is reduced and the backfat thickness is increased as the concentrations of dietary peas are increased (Stein et al., 2006). However, it was also demonstrated that this situation may be ameliorated by including crystalline Met and Trp in the diets. In other research showed that inclusion of 35% peas as a substitute for soybean meal had no detrimental effects on nutrient digestibility, pig performance and carcass traits (Castell et al., 1988), but in trial of researchers Gatel and Grosjan, (1990), the use of pea greater than 20% as alternative protein source in pig diets reduced performance.

There were no differences of ash content and pH in *Longissimus dorsi* muscle, but moisture, fat, crude protein and cholesterol content were significantly different between soybean and peas groups (Table 4). Fat content in *Longissimus dorsi* muscle was 2.5 till 2.8% higher (p<0.01) for peas group pigs than of soybean pigs group.

The similar results was found in research were peas included from 36–66% in pig groups diets, the pH and marbling of the *Longissimus dorsi* muscle and 10th rib backfat thickness were not influenced by treatment (Stein et al., 2006).

Interest in meat fatty acid composition stems mainly from the need to produce healthier meat, i.e., meat with higher ratio of polyunsaturated (PUFA) to saturated fatty acids and a more favourable balance between n-6 and n-3 PUFA. In pigs, the drive has been to increase n-3 PUFA in meat and this can be achieved by feeding sources.

### Table 4

**Characteristics of *Longissimus dorsi* muscle (n=12)**

<table>
<thead>
<tr>
<th>Indices</th>
<th>1, 2 soybean groups</th>
<th>1 peas group</th>
<th>2 peas group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture, %</td>
<td>72.3±0.47</td>
<td>70.1±0.43</td>
<td>69.2±0.55</td>
</tr>
<tr>
<td>Fat, %</td>
<td>4.4±0.42</td>
<td>6.9±0.21</td>
<td>7.2±0.06</td>
</tr>
<tr>
<td>CP, %</td>
<td>24.2±0.13</td>
<td>22.9±0.35</td>
<td>22.1±0.15</td>
</tr>
<tr>
<td>Ash, %</td>
<td>1.27±0.09</td>
<td>1.2±0.06</td>
<td>1.13±0.03</td>
</tr>
<tr>
<td>pH</td>
<td>5.45±0.02</td>
<td>5.39±0.01</td>
<td>5.24±0.01</td>
</tr>
<tr>
<td>Cholesterol, mg 100 g</td>
<td>108.4±0.29</td>
<td>105.0±0.54</td>
<td>81.3±0.23</td>
</tr>
</tbody>
</table>

Values are presented as mean ±standard error. a,b,c,d,e p<0.05; b,d,f,g p<0.01; k p<0.001

Only when concentration of alpha-linolenic (C18:3) approach 3% of neiral lipids or phospholipids are there any adverse effects on meat quality and flavour (Wood et al., 2003). Several papers have examined the effects of dietary oils containing a high proportion of 18:2 on the acid composition and quality of pigmeat, for example soya oil maize and sunflower, but not a peas. In present research the fatty acid content were not high, but were significantly (p<0.001, p<0.01, p<0.05) different with pigs of soybean groups (Table 5). High levels of PUFA are undesirable in pork, because they adversely affect consistency, storage stability and texture of the processed pork products.

The amino acids content was determined only in one sample of muscle from each pig group because the price of analyses was very high. The measured values of selected amino acids with different feeding in the groups are shown in Table 6.
The highest values in the group of nonessential amino acids were measured in glutamic (3.09–3.54 g), aspartic acid (1.31–1.99%) and leucine (1.19–1.70 g). The content of lysine was from 1.21 till 1.98 g. The lowest value were found out in the amino acids tryptophane (0.245–0.274 g) and proline (0.37 g) in soybean group, but in peas groups the content of proline was (0.772–0.779 g) and was more twice times higher than tryptophan. The biological value of meat is defined by fully valuable protein (Jukna et al., 2005). The full value of meat protein is calculating according to the relationship of irreplaceable amino acid tryptophan, which is found only in muscle tissue, and replaceable amino acid hydroxyproline, found only in connective tissue. Higher ratio shows more biologically valuable meat protein. The ratio tryptophan: hydroxyproline in pork was 7.9 (Ribikauskiene, 2003), but data in Table 6 evidently demonstrate that peas groups pork contained more proline than tryptophan.

**Conclusions**

The study indicates that 15 and 28% inclusion of pea in finisher pig diets about 3–5% increase liveweight gain. The higher pig daily gain resulted on the increasing of fat in the body at the similar feed consumption per 1 kg liveweight gain.

Carcass indicators show a tendency to accumulate more fat tissue in pigs, which fed pea in the feed. The internal fat about 0.68 and 0.48 kg were more with significant differences (p<0.001) between soybean pig groups. For pigs, which fed peas, average backfat were of 14.3 mm, 2 and 1.3 mm more than the pigs in soybean groups.

There were no differences of ash content and pH in *Longissimus dorsi* muscle, but moisture, fat, crude protein and cholesterol content were significantly different between soybean and peas groups. Fat content in *Longissimus dorsi* muscle was 2.5 till 2.8% higher (p<0.01) for peas group pigs.

The fatty acids content in peas pig groups were not high, but were significantly (p<0.001, p<0.01, p<0.05) different with pigs of soybean groups.

The highest values in the group of nonessential amino acids were measured in glutamic (3.09–3.54 g), The lowest value were found out in the amino acids tryptophane (0.245 g) and proline (0.37 g) in soybean group, but in peas groups the content of proline were (0.772–0.779 g) and was more twice times higher than tryptophan.

The peas may replace soybean meal in diets fed to finishing pigs without negatively affecting pig performance that provided diets are balanced for concentrations of digestible indispensable amino acids.

**Acknowledgment**

The research was done on financial support of Project Nr. K33 “Pulses as alternative protein feeds instead the soybean meal, its production, agricultural and economic evaluation in Latvia conditions”

**References**


ANTIMICROBIAL SUSCEPTIBILITY OF OXACILLIN-RESISTANT STAPHYLOCOCCUS SPP. ISOLATED FROM Poultry PRODUCTS

Modestas Ružauskas¹, Lina Vaškevičiūtė¹, Rita Šiugždinienė¹, Irena Klimienė¹, Marius Virgailis¹, Rita Plančiūnienė¹, Lina Anskienė², Raimundas Mockeliūnas³, Alvydas Pavilonis¹

¹Microbiology and Virology Institute, Lithuanian University of Health Sciences, Tiltės 18, Kaunas, Lithuania, e-mail: ruzauskas@lva.lt
²Department of Animal Reproduction, Veterinary Academy, Lithuanian University of Health Sciences, Tiltės 18, Kaunas, Lithuania

Abstract

The aim of this study was to investigate the prevalence of staphylococci in raw poultry products intended for human consumption and to determine antimicrobial susceptibility, particularly of oxacillin-resistant isolates. Poultry (chicken) liver as representative samples were randomly selected in different retail markets. Isolation of staphylococci was performed using general and selective nutrient media including Mannitol Salt Agar supplemented with cefoxitin as well as Brilliance MRSA2 Agar. Antimicrobial susceptibility testing was carried out using “Sensititre” plates for determination of minimal inhibitory concentrations. Interpretation of results was performed according to CLSI standard. Polymerase chain reaction was used for determination of mec genes.

Fifty samples from 70 tested were positive for Staphylococcus spp. (71%). In 30% of the isolated staphylococci, the growth was observed on selective media with cefoxitin as well as on MRSA2 Agar. The isolates from those samples were resistant to oxacillin (MIC≥0.5 mg L⁻¹) however, the only one isolate harboured the mecA gene. All of the isolates with phenotypic resistance to oxacillin were susceptible to vancomycin, gentamicin, linezolid, daptomycin, rifampin and quinupristin/dalfopristin. The resistances of those isolates were observed to tetracycline (60%), erythromycin (40%), and fluoroquinolones (40%). The species of oxacillin-resistant staphylococci included S. hyicus (6), S. hominis (4), S. haemolyticus (1), S. cohnii (1), S. lentus (1), S. warneri (1) and S. intermedius (1). Oxacillin-resistant Staphylococcus aureus was not found. The obtained results demonstrated low correlation between phenotypic resistance to oxacillin and the presence of mec genes in staphylococcal isolates from the poultry products. Further studies need to be performed for investigation of this unusual finding.

Keywords: Staphylococcus, mecA, resistance, oxacillin, poultry.

Introduction

Staphylococcus spp. are significant bacteria in the etiology of avian diseases and may thus contaminate foods as a result of processed carcasses (Mead, Dodd, 1990; Pepe et al., 2006). Although enterotoxin-producing S. aureus is the most common cause of food-borne human illness throughout the world (Do Carmo et al., 2004; LeLoir et al., 2003) the other species such as S. hyicus, S. sciuri, S. xylosus or S. cohnii are also important, particularly because of carriage the genes encoding antimicrobial resistance (Aarestrup et al., 2000; Kawano et al., 1996). While staphylococci commonly occur on the skin and nasopharynx of healthy poultry (Mead, Dodd, 1990), they can survive, colonize, and persist at various processing stages in commercial poultry processing plants due to the expression of various key properties, including adhesion (Chaffey, Waites, 1987). Staphylococci are one of the most predominant groups during the slaughtering and processing of poultry, and they have been recovered from air samples (Ellerbroek, 1997), neck skin of chicken carcasses (Geornaras et al., 1995; Olivier et al., 1996), and machinery surfaces (Mead et al., 1995; Huys et al., 2005). By this reason contaminated poultry products could be the source of possible transmission of different staphylococci species including resistant strains to humans, during food processing at home. The aim of this study was to investigate the prevalence of staphylococci in raw poultry products intended for human consumption, to determine species distribution and antimicrobial susceptibility, particularly of oxacillin-resistant isolates.

Materials and Methods

In 2013 seventy samples of raw poultry liver as representative samples intended for human consumption were randomly selected on different retail markets in Lithuania. The samples were delivered to the laboratory during 1-3 hours. Material was soaked into technically sterile plastic bags containing sterile Tryptone-Soya Broth (Oxoid, Thermo Fisher). Sterile cotton swabs were used for inoculation of outwash onto Sheep Blood Agar, Mannitol Salt Agar (Liofilchem, Italy), Mannitol Salt Agar supplemented with 4 mg L⁻¹ cefoxitin (Sigma-Aldrich), Brilliance MRSA 2 Agar (Oxoid, Thermo Scientific) as well as on Contrast MRSA Broth (Oxoid, Thermo Fisher). Staphylococci up to the genus level were identified according to morphology characteristics, catalase production, gram-staining, susceptibility to furazolidone. PCR was used for identification of genus specific (16S) subunit. DNA material for molecular testing was obtained after bacterial lysis according to the extraction protocol prepared by the Community Reference Laboratory for Antimicrobial Resistance (Anonymous, 2009) with slight modifications. Briefly, bacterial colonies were taken with a bacteriological loop from the surface of Mueller Hinton Agar and transferred to phosphate buffered saline (pH 7.3). The content was centrifuged for 5 min at 13 000 rpm. Then the supernatant was discarded and the pellet was re-suspended in Tris-EDTA (TE) buffer. The suspension was heated using thermomixer (Biosan) in 100 °C degrees for 10 minutes. Boiled suspension was transferred directly on ice and diluted by 1:10 in TE. Species identification was performed according to pigment and coagulase production, presence of protein...
A and clumping factor as well as on biochemical properties using RapID Staph Plus (Thermo Scientific) identification system. In complicated cases species determination was performed by 16S rRNA sequencing using ABI3730XL sequencer. The primers for obtaining 16S rRNA product (1405 bp) as well as for sequencing were used as follows: forward - 5’ GCTCAGGA(C/T)GAAACGCTGG 3’ and the reverse - 5’ AGACGATCCWTCAGTGAGC 3’ (Couto, 2014). Sequences were analysed using Molecular Evolutionary Genetic Analysis software (MEGA, version 6). Basic local alignment search tool (BLAST) was used for comparison of obtained sequences with sequences presented in the database of National Center of Biotechnology Information.

Antimicrobial susceptibility testing was performed using the broth microdilution method. Sensititre® plates and ARIS 2X automated system (Thermo Scientific) were used with the following antimicrobials: daptomycin, ciprofloxacin, clindamycin, erythromycin, gentamicin, levofloxacin, linezolid, oxacillin, penicillin, co-trimoxazole, quinupristin/dalfopristin and rifampin. Interpretation of results was carried-out using manufacturers software (SWIN®) adapted to clinical breakpoints of Clinical and Laboratory Standards Institute (CLSI). The quality control strain S. aureus ATCC 29213 was included in each assay for validation purposes. Detection of mecA and mecA/LGA25 (mecC) genes was performed by PCR described previously (Cuny et al., 2011; Anonymous, 2009).

Statistical analysis was performed using Microsoft Excel software. Comparison between categorical variables was calculated by chi-square and Fisher’s exact test. Results were considered statistically significant if p<0.05.

**Results and Discussion**

Fifty samples from 70 tested were positive for *Staphylococcus* spp. (71%). In 30% of isolated staphylococci the growth was observed on Mannitol Salt Agar supplemented with cefoxitin as well as on MRSA2 Agar and/or Contrast MRSA Broth. The isolates from those samples were resistant to oxacillin (MIC≥0.5 mg L⁻¹) however the only one isolate harboured the mecA gene. All of the isolates phenotypically resistant to oxacillin were susceptible to vancomycin, gentamicin, rifampin, linezolid, daptomycin and quinupristin/dalfopristin. 40% of the isolates demonstrated resistance to at least one fluoroquinolone and erythromycin – the antimicrobials that are treated as critically important for humans. Attention should be paid to this unfavourable finding although it is in coincidence with our previous studies, where high frequency of resistance in other bacterial species (enterococci and *Escherichia coli*) isolated from poultry products has been detected toward fluoroquinolones and/or macrolides (Ružauskas et al., 2010a; Ružauskas et al., 2010b). Resistance to tetracycline was frequent and reached 60%. There are different data associated with *Staphylococcus* spp. isolated from poultry according to the resistance to tetracycline. For example, Shareef et al. (2009) found that 100% of the isolated *S. aureus* from layers were susceptible to tetracycline, while Jevinova et al., (2009) found that 22% of the *Staphylococcus* spp. from poultry meat demonstrated resistance to this antibiotic. The distributions of minimal inhibitory concentration (MIC) of the oxacillin-resistant isolates are presented in Table 1.

<table>
<thead>
<tr>
<th>MIC distributions (%) (mg L⁻¹)</th>
<th>0.12</th>
<th>0.25</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>16</th>
<th>32</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIP</td>
<td>67</td>
<td>0</td>
<td>0</td>
<td>33</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLI</td>
<td>47</td>
<td>20</td>
<td>7</td>
<td>13</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DAP</td>
<td>7</td>
<td>73</td>
<td>20</td>
<td>7</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ERY</td>
<td>0</td>
<td>60</td>
<td>0</td>
<td>0</td>
<td>40</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAT</td>
<td>74</td>
<td>13</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GEN</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LEV</td>
<td>47</td>
<td>20</td>
<td>0</td>
<td>7</td>
<td>13</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LZD</td>
<td>13</td>
<td>40</td>
<td>47</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OXA</td>
<td>0</td>
<td>53</td>
<td>33</td>
<td>7</td>
<td>0</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEN</td>
<td>40</td>
<td>27</td>
<td>20</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SYN</td>
<td>0</td>
<td>13</td>
<td>47</td>
<td>20</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RIF</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TET</td>
<td>33</td>
<td>7</td>
<td>60</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SXT</td>
<td>86</td>
<td>7</td>
<td>7</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAN</td>
<td>93</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Grey cells – susceptible; white cells – intermediate; dark cells – resistant


The species of oxacillin-resistant staphylococci included *S. hyicus* (6), *S. hominis* (4), *S. haemolyticus* (1), *S. cohnii* (1), *S. lentus* (1), *S. warneri* (1) and *S. intermedius* (1). Oxacillin-resistant *Staphylococcus aureus* was not found. Identification of the aforementioned species using biochemical testing was reliable only for 60% of the isolates. For remaining cases sequencing of 16S rRNA was necessary for taxonomic verification.

The obtained results demonstrated low correlation (p>0.05) between phenotypic resistance to oxacillin and the presence of mecA gene in staphylococcal isolates from the poultry products. Recently Ba with co-authors (2014) found the similar findings in phenotypically oxacillin-resistant isolates of *Staphylococcus aureus*. Those strains didn’t carried neither mecA nor mecC genes. The authors identified a number of amino acid substitutions present in the
endogenous penicillin binding proteins (PBP) in the resistant strains that were absent in closely related methicillin-susceptible strains. Possibly that substitutions of PBP could be the basis of resistance expression. There are no clear data about similar PBP in other Staphylococcus species up to date however, our findings suggest that similar penicillin binding proteins might be expressed in other Staphylococcus species as well. Interestingly, in 35 isolates of staphylococci (both coagulase-positive (CPS) and coagulase-negative (CNS) species) isolated from dogs recently (data not presented) we detected mecA gene in all of the isolates phenotypically resistant to oxacillin. However, the important aspect for the absence of mecA gene in some of the isolates in this study might be associated with different Staphylococcus species, where both CNS and CPS are presented. CLSI as well as European Committee on Antimicrobial Susceptibility Testing set oxacillin breakpoint as >0.25 mg L\(^{-1}\) only for CNS species, whereas some CPS species had no interpretation criteria. In any case, the results of this study are interesting, therefore further studies need to be performed for the investigation of this unusual finding associated with the absence of mec genes in oxacillin-resistant staphylococci prevalent in raw poultry products. Oxacillin-resistant species prevalence in poultry products is interesting as well as some of the species including S. haemolyticus, S. hominis and S. cohnii are prevalent in humans and are well-known because of the high antimicrobial resistance. This fact should be taken into account for safe food production.

Conclusions

Oxacillin-resistant staphylococci are prevalent in retail poultry products in Lithuanian markets, although oxacillin-resistant S. aureus has not yet been detected. The obtained results demonstrated low correlation between phenotypic resistance to oxacillin and the presence of mec genes in staphylococcal isolates from the poultry products. All oxacillin-resistant staphylococci were susceptible to vancomycin, gentamicin, linezolid, daptomycin, rifampin, co-trimoxazole and streptogramins. Resistance was observed toward fluoroquinolones (40%), macrolides (40%) and tetracycline (60%). Human-associated Staphylococcus species – S. haemolyticus, S. hominis and S. cohnii are prevalent in retail poultry products as well.

Acknowledgment

This research was funded by a grant (MIP-075/2013) from the Research Council of Lithuania.

References


DETERMINATION OF THE CONTENT OF COENZYMES Q₉ AND Q₁₀ IN PORK MEAT FROM DIFFERENT BREEDS

Sónia C. Andrade¹, Raquel P. F. Guiné¹,², Luisa C. P. Roseiro³

¹ Department Indústrias Alimentares – Escola Superior Agrária de Viseu, Viseu, Portugal, e-mail: soniandrade89@hotmail.com
² CI&DET/ Dep. Indústrias Alimentares – Escola Superior Agrária de Viseu, Viseu, Portugal
³ Instituto Nacional de Investigação Agrária e Veterinária, Lisboa, Portugal

Abstract

Meat contains basic and essential nutrients to humans, and recently has been given a special attention to the presence of bioactive compounds. The objective of this work was the simultaneous determination of coenzymes Q₉ and Q₁₀ in pork meat, using HPLC. For this study were used 122 meat samples from different muscles and different breeds of pork. Besides, it was also evaluated the influence of animal breed and muscle type in the contents of CoQ₉ and CoQ₁₀. The results showed that the breed influenced significantly the mean content of CoQ₉ (0.70 mg 100 g⁻¹) and CoQ₁₀ (3.76 mg 100 g⁻¹) (p<0.0001), being this significantly higher in the meat from the breed ‘Alentejano’ when compared to the other breeds (Large White and Landrace). The type of muscle was also responsible for the variation in the levels of coenzymes in the samples analysed (p<0.05) and (p <0.0001) respectively. From the results obtained it was concluded that the breed ‘Alentejano’ presented the highest contents in both coenzymes (CoQ₉ and CoQ₁₀) and that, among the muscles evaluated, the muscle Seminembranosus (Sm) showed higher concentrations of CoQ₁₀.

Keywords: chromatographic analysis, coenzyme Q₉, coenzyme Q₁₀, pork meat.

Introduction

The negative image often associated with meat and processed meat products is related to the presence of some components that can cause diseases to the health of the consumer, particularly high salt content, saturated fats, fatty acids and cholesterol responsible for the development of cardiovascular diseases, some cancers and obesity (Leunceford, 2008). Meat also plays an important role in supplying our diet with minerals, such as iron, zinc, selenium, and vitamins of group B and E. Besides these basic nutritional components, studies have revealed that meat contains several bioactive compounds, such as conjugated linoleic acid (CLA), L-carnitine, carnosine, glutathione, taurine, creatine, coenzyme Q₉ and Q₁₀, among others (Toldrá, 2010).

The coenzymes Q contain a chromogenic nucleus (2,3-dimethoxy-5-methyl-1,4-benzoquinone) and a polyisoprenoid side chain in the 6-position which differs only in the number of isoprenoid units (Souchet, Laplante, 2007). The predominant form of ubiquinone in humans, animals, and fish is Co-enzyme Q₁₀ (2,3-dimethoxy-5-methyl-6-decaprenylbenzoquinone-ne), containing 10 isoprenoid units in the side chain (Linnane, 2007).

Coenzyme Q is an essential component of the electron transport chain electrons respiratory complexes I and II to respiratory complex III. Another important function of coenzyme Q is that of serving as a lipid soluble antioxidant protecting lipids, proteins and DNA from damage by reactive oxygen species (Santos, 2011).

In the respiratory chain in the mitochondria there are several coenzymes (coenzyme Q), as for example, CoQ₂, CoQ₃, CoQ₄, CoQ₅, CoQ₆, CoQ₇, CoQ₈, CoQ₉, and CoQ₁₀. The Q₁₀ is the most common form found in humans and most mammals, while coenzyme Q₉ is the most often found in rats and guinea pig. On the other hand, the Q₄, Q₇ and Q₈ coenzymes are found in bacteria and yeasts (Sauer Shah, Laurindo, 2010).

The presence of coenzymes Q₉ and Q₁₀ in meat assumes a particular importance given their important roles in the human body as promoting health. However, the contents of these coenzymes may vary greatly according to breed, muscle type, sex, age, seasonal variations, and type of feed, among others. Given the importance of natural antioxidants as preventing the damaging effect of free radicals formed during the cellular processes that are responsible for e.g. aging, cardiovascular disease and certain cancers, the objective of this work was to determine the content of coenzymes Q₉ and Q₁₀ in several samples of pork meat from different breeds by the method of HPL.

Materials and Methods

Sampling

In this study were used samples taken from meat originating from different animal breeds and different muscles. The pork samples corresponded to meat from 61 animals, from different breeds: Large White × Landrace (31 animals) and ‘Alentejana’ (30 animals). From each animal, samples of muscles Longissimus dorsi (Ld) and semimembranosus (Sm) were removed, summing up a total of 122 samples analyzed.

The samples were homogenized and defatted, vacuum packed and kept frozen at -72 °C to perform the analysis.

Analytical Process

All the methodology inherent in the study and analysis of pork meat from different breeds was based on a technical protocol previously defined by Section of Meat and Meat Products from the National Institute for Agricultural and Veterinary Research (INIAV, 2013). These procedures were previously adapted to the study of meat and used for the analyses.
**Extraction**

For extraction were weighed 4 g of homogenized muscle sample, and cut into small pieces into glass petri dishes, in duplicate. Then, the Petri dishes were placed for about 24 hours in the lyophilizer (CHRIST BETA 1-8 K). The lyophilized samples were reduced to powder in a mortar placed in plastic centrifuge tube with cap and kept in a dry place until analysis. To the sample is added 10 mL of 0.15 M NaCl solution and then homogenized in a homogenization equipment (Polytron PT 3100) for 1 minute at 3500 rpm. After homogenization, was added 10 mL of absolute ethanol and stirred by vortex for 2 minutes (Vortex Heidolph REAX). Then, to the samples were added 15 mL of n-hexane and centrifuged again for 1 minute (Centrifuge Sorvall Instruments RC5C with SS-34 rotor). The extracts were then centrifuged at 3000 rpm at 4 °C for 5 minutes to separate the phases. The supernatant was removed to another tube, properly identified, and proceeded to re-extraction of the pellet with another 15 mL of n-hexane, followed by further centrifugation. The supernatants from both centrifugations were combined. From this extract, 20 mL were used to evaporate on a rotary evaporator at 40 °C to dryness. The residue obtained was resuspended in 3 mL of 2-propanol and filtered through a syringe of 0.2 mm equipped with a PTFE filter (Acrodisc). Fifty microliters were injected into the HPLC system.

**Analysis by high performance liquid chromatography (HPLC)**

A Waters HPLC system consisting of a mark separation module Alliance 2487 and a detector Waters 2695 was used for the analyses. Empower Pro software was used to analyze the chromatograms obtained. The separation of coenzymes Q\textsubscript{9} and Q\textsubscript{10} was performed using an isocratic phase column Atlantis dC 18 4.6×150 mm. 3 μm. Waters mark at room temperature.

The mobile phase consisting of acetonitrile: tetrahydrofuran: water (55 : 40 : 5, v/v/v) was used at a flow rate of 1.5 mL min\textsuperscript{-1}. The coenzymes were detected in UV detector at 275 nm.

**Analysis of Results**

To evaluate the influence of the studied factors (breed and muscle) and their interactions on the variation of the levels of coenzyme Q\textsubscript{9} and Q\textsubscript{10} there an Analysis of Variance was undertaken for 95% confidence level. When differences were significant for the race factor or interactions, the difference between means was assessed using the Tukey test.

The following factors were assessed:

- Influence of breed in the content of coenzymes Q\textsubscript{9} and Q\textsubscript{10}.
- Influence of muscle (Ld and Sm) in the content of coenzymes Q\textsubscript{9} and Q\textsubscript{10}.
- Influence of the interaction between muscle and breed.

**Results and Discussion**

**Validation of analytical procedures**

**Determination of standard curves coenzymes Q\textsubscript{9} and Q\textsubscript{10}**

In order to proceed to quantification of the content of coenzymes in the samples, were prepared two solutions of coenzyme Q\textsubscript{9} and Q\textsubscript{10}, which were injected into the HPLC in the form of various concentrations 0, 2, 5, 10, 20, 30, 50 (μg mL\textsuperscript{-1}), giving rise to the various “peaks” of different heights (AU), being the detection time 6.095 minutes to Q\textsubscript{9} and 8.065 minutes to Q\textsubscript{10}.

With the integrations of “peaks” and the respective known concentrations of coenzymes, it was possible to obtain both standard curves, with their corresponding equations and correlation coefficients (r).

**Determination of the Limits of Detection (LOD) and Quantification (LOQ)**

The LOD and LOQ were determined by a sequence of analytical calculations to determine the standard deviation(s), using the specific formulas, from which were obtained the values in Table 1.

<table>
<thead>
<tr>
<th>Type of coenzyme</th>
<th>LOD</th>
<th>LOQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coenzyme Q_9, mg 100 g\textsuperscript{-1}</td>
<td>0.343</td>
<td>1.040</td>
</tr>
<tr>
<td>Coenzyme Q_{10}, mg 100 g\textsuperscript{-1}</td>
<td>0.067</td>
<td>0.203</td>
</tr>
</tbody>
</table>

**Determination of recovery rate**

The calculus of the recovery rate is done using the peak obtained for the sample with the standard and the peak of the sample without the standard. This determination is made by means of the expression for the calculus of the recovery rate.

The results of the recovery rates made can be seen in Table 2.

<table>
<thead>
<tr>
<th>Type of coenzyme</th>
<th>Recovery Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coenzyme Q_9, mg 100 g\textsuperscript{-1}</td>
<td>50.00</td>
</tr>
<tr>
<td>Coenzyme Q_{10}, mg 100 g\textsuperscript{-1}</td>
<td>69.77</td>
</tr>
</tbody>
</table>

**Simultaneous determination of coenzymes Q\textsubscript{9} and Q\textsubscript{10} in pork meat**

**Coenzyme Q\textsubscript{9}**

Statistical analysis undertaken to the results of the determination of coenzyme Q\textsubscript{9} in pork meat, demonstrated significant differences between the breeds studied (p<0.0001) and between muscles Ld and Sm (p <0.05). With respect to the interaction between the two factors (Breed × Muscle) it was not found any significant influence.

Among the factors studied, the breed of the animal was that which exerted a significant influence over the content of coenzyme Q\textsubscript{9} in pork meat, with F value equal to 27.25.
According to Figure 1, it was possible to assess that there is a significant difference in the mean values (p<0.001). The samples obtained from animals of ‘Alentejana’ breed showed an average content of coenzyme Q₉ higher (0.70±0.21 mg 100 g⁻¹) than samples of the animals LW×LR (0.52±0.19 mg 100 g⁻¹).

With respect to the influence of the muscle type in the amount of Coenzyme Q₉, it was found a significant difference between the two muscles in the study (Ld and Sm). The Sm muscle showed a higher average (0.64±0.23 mg 100 g⁻¹), when compared with Ld muscle (0.57±0.21 mg 100 g⁻¹) (Figure 1).

Through the second graph of Figure 1, it was possible to ascertain that the average value for Sm CoQ₉ was significantly higher than Ld. A possible reason for this difference between the two types of muscle is due to the fact that muscle Sm belongs to the leg muscles, and the muscles in this location exert a greater physical effort for the animal’s mobility.

The interaction between breed and muscle showed no significant influence on the results of coenzyme Q₉ in pork meat.

Coenzyme Q₁₀
In analyzing the results (ANOVA) of the content of CoQ₁₀ in pork meat, it was found that all the factors studied: race, muscle, interaction of breed with the type of muscle, significantly influence the content of CoQ₁₀ (p<0.0001).

Regarding the race factor, it was obtained a value of F equa to 226.78. In the first graph of Figure 2 are presented the average values for coenzyme Q₁₀ in meat from breeds ‘Alentejana’ (3.75±1.44 mg 100 g⁻¹) and LW×LR (1.96±0.75 mg 100 g⁻¹). The results demonstrated that it is the meat of the ‘Alentejana’ breed that has a higher content compared with that obtained in pork LW×LR (white pig) (Figure 2).

The muscle factor also exerted a high influence on the result, with a value of F equal to 213.46. It was also found that the Sm muscle (3.70±1.53 mg 100 g⁻¹) showed a higher mean value compared to Ld muscle (1.96±0.66 mg 100 g⁻¹). The difference between the two types of muscle examined can be observed in the second graph in Figure 2.

This difference that occurred between muscles, as already mentioned in the analysis of CoQ₉, may be due to the type of muscle and breed. In fact, the animal exerts more physical effort with some of their muscles, which causes them to gain a greater oxidative character.

Doing the statistical analysis regarding the interaction of breed with muscle it was found to be a high-examined significant difference (p<0.0001), with value F =37.0.

In analyzing the results (ANOVA) of the content of CoQ₁₀ in pork meat, it was found that all the factors studied: race, muscle, interaction of breed with the type of muscle, significantly influence the content of CoQ₁₀ (p<0.0001).

Regarding the race factor, it was obtained a value of F equa to 226.78. In the first graph of Figure 2 are presented the average values for coenzyme Q₁₀ in meat from breeds ‘Alentejana’ (3.75±1.44 mg 100 g⁻¹) and LW×LR (1.96±0.75 mg 100 g⁻¹). The results demonstrated that it is the meat of the ‘Alentejana’ breed that has a higher content compared with that obtained in pork LW×LR (white pig) (Figure 2).

The muscle factor also exerted a high influence on the result, with a value of F equal to 213.46. It was also found that the Sm muscle (3.70±1.53 mg 100 g⁻¹) showed a higher mean value compared to Ld muscle (1.96±0.66 mg 100 g⁻¹). The difference between the two types of muscle examined can be observed in the second graph in Figure 2.

With respect to the influence of the muscle type in the amount of Coenzyme Q₉, it was found a significant difference between the two muscles in the study (Ld and Sm). The Sm muscle showed a higher average (0.64±0.23 mg 100 g⁻¹), when compared with Ld muscle (0.57±0.21 mg 100 g⁻¹) (Figure 1).

Through the second graph of Figure 1, it was possible to ascertain that the average value for Sm CoQ₉ was significantly higher than Ld. A possible reason for this difference between the two types of muscle is due to the fact that muscle Sm belongs to the leg muscles, and the muscles in this location exert a greater physical effort for the animal’s mobility.

The interaction between breed and muscle showed no significant influence on the results of coenzyme Q₉ in pork meat.

Coenzyme Q₁₀
In analyzing the results (ANOVA) of the content of CoQ₁₀ in pork meat, it was found that all the factors studied: race, muscle, interaction of breed with the type of muscle, significantly influence the content of CoQ₁₀ (p<0.0001).

Regarding the race factor, it was obtained a value of F equa to 226.78. In the first graph of Figure 2 are presented the average values for coenzyme Q₁₀ in meat from breeds ‘Alentejana’ (3.75±1.44 mg 100 g⁻¹) and LW×LR (1.96±0.75 mg 100 g⁻¹). The results demonstrated that it is the meat of the ‘Alentejana’ breed that has a higher content compared with that obtained in pork LW×LR (white pig) (Figure 2).

The muscle factor also exerted a high influence on the result, with a value of F equal to 213.46. It was also found that the Sm muscle (3.70±1.53 mg 100 g⁻¹) showed a higher mean value compared to Ld muscle (1.96±0.66 mg 100 g⁻¹). The difference between the two types of muscle examined can be observed in the second graph in Figure 2.

With respect to the influence of the muscle type in the amount of Coenzyme Q₉, it was found a significant difference between the two muscles in the study (Ld and Sm). The Sm muscle showed a higher average (0.64±0.23 mg 100 g⁻¹), when compared with Ld muscle (0.57±0.21 mg 100 g⁻¹) (Figure 1).

Through the second graph of Figure 1, it was possible to ascertain that the average value for Sm CoQ₉ was significantly higher than Ld. A possible reason for this difference between the two types of muscle is due to the fact that muscle Sm belongs to the leg muscles, and the muscles in this location exert a greater physical effort for the animal’s mobility.

The interaction between breed and muscle showed no significant influence on the results of coenzyme Q₉ in pork meat.

Coenzyme Q₁₀
In analyzing the results (ANOVA) of the content of CoQ₁₀ in pork meat, it was found that all the factors studied: race, muscle, interaction of breed with the type of muscle, significantly influence the content of CoQ₁₀ (p<0.0001).

Regarding the race factor, it was obtained a value of F equa to 226.78. In the first graph of Figure 2 are presented the average values for coenzyme Q₁₀ in meat from breeds ‘Alentejana’ (3.75±1.44 mg 100 g⁻¹) and LW×LR (1.96±0.75 mg 100 g⁻¹). The results demonstrated that it is the meat of the ‘Alentejana’ breed that has a higher content compared with that obtained in pork LW×LR (white pig) (Figure 2).

The muscle factor also exerted a high influence on the result, with a value of F equal to 213.46. It was also found that the Sm muscle (3.70±1.53 mg 100 g⁻¹) showed a higher mean value compared to Ld muscle (1.96±0.66 mg 100 g⁻¹). The difference between the two types of muscle examined can be observed in the second graph in Figure 2.

With respect to the influence of the muscle type in the amount of Coenzyme Q₉, it was found a significant difference between the two muscles in the study (Ld and Sm). The Sm muscle showed a higher average (0.64±0.23 mg 100 g⁻¹), when compared with Ld muscle (0.57±0.21 mg 100 g⁻¹) (Figure 1).

Through the second graph of Figure 1, it was possible to ascertain that the average value for Sm CoQ₉ was significantly higher than Ld. A possible reason for this difference between the two types of muscle is due to the fact that muscle Sm belongs to the leg muscles, and the muscles in this location exert a greater physical effort for the animal’s mobility.

The interaction between breed and muscle showed no significant influence on the results of coenzyme Q₉ in pork meat.

Coenzyme Q₁₀
In analyzing the results (ANOVA) of the content of CoQ₁₀ in pork meat, it was found that all the factors studied: race, muscle, interaction of breed with the type of muscle, significantly influence the content of CoQ₁₀ (p<0.0001).

Regarding the race factor, it was obtained a value of F equa to 226.78. In the first graph of Figure 2 are presented the average values for coenzyme Q₁₀ in meat from breeds ‘Alentejana’ (3.75±1.44 mg 100 g⁻¹) and LW×LR (1.96±0.75 mg 100 g⁻¹). The results demonstrated that it is the meat of the ‘Alentejana’ breed that has a higher content compared with that obtained in pork LW×LR (white pig) (Figure 2).

The muscle factor also exerted a high influence on the result, with a value of F equal to 213.46. It was also found that the Sm muscle (3.70±1.53 mg 100 g⁻¹) showed a higher mean value compared to Ld muscle (1.96±0.66 mg 100 g⁻¹). The difference between the two types of muscle examined can be observed in the second graph in Figure 2.
Among all the conditions studied, the meat samples belonging to the Sm muscle of ‘Alentejana’ pig breed (Figure 3), exhibited a higher average value. The mean value obtained for the samples of coenzyme Q_{10} in Sm muscle of the ‘Alentejana’ breed was 4.99 mg 100 g^{-1}. The values obtained for meat samples of Sm muscle from LW×LR breed and the values obtained for meat samples of Ld muscle from the ‘Alentejana’ breed showed no significant difference (Figure 3). The Ld samples of animals from LW×LR breed showed the lowest mean level of CoQ_{10} (1.44 mg 100 g^{-1}), also revealing to be different from the medium content of all other conditions studied (Figure 3).

It was further observed that the highest values for CoQ_{9} and CoQ_{10} occurred in the most oxidative muscle (Sm), which belongs to the leg muscle, where a greater physical effort is done having in mind the mobility of the animal. It is also known that the higher is the oxidative nature of the muscle, the largest will be the content of coenzymes Q_{9} and Q_{10}, as found in this study.

Among the breeds analyzed that one which stood out for its high content of coenzyme Q_{10} was the sample of swine of the ‘Alentejana’ breed.

**Conclusion**

This study aimed to determine the coenzymes Q_{9} and Q_{10} in different meat samples. In view of the results obtained, it was evaluated the influence in the concentration of the coenzymes of the factors under study (breed, muscle and interaction between the two). As regards pork meat, the results indicated that factors like breed and type of muscle exerted an influence on the content of coenzyme Q_{9}. However in the case of CoQ_{10} all the factors studied revealed to have a significant influence.

**References**

THE EFFECTS OF pH, a_w, AND LACTIC ACID BACTERIA ON LISTERIA MONOCYTOGENES IN FERMENTED SAUSAGES

Indulis Silins
Institute of Food and Environmental Hygiene, Faculty of Veterinary Medicine, Latvia University of Agriculture, Helmana street 8, Jelgava, Latvia, e-mail: indulis.sil@inbox.lv

Abstract
The survival of inoculated in fermented sausages Listeria monocytogenes strain was studied. The sausages were prepared with and without starter cultures. The survival limits of L monocytogenes  and lactic acid bacteria (LAB) were determined as colony forming units per gram (cfu g⁻¹) depending on water activity (a_w) and pH on 0, 1st, 3rd, 5th, 7th, 14th and 21st days of maturation. The decreasing water activity conditioned by moisture (weight) loss during ripening and pH decrease ensured negative polynomial growth rate of inoculated L. monocytogenes -0.27 lg (cfu g⁻¹) each day of ripening time, and -0.65 lg (cfu g⁻¹) on the first 7 days of maturation. A significant Pearson’s correlation (p<0.01) was established between decreased values of L. monocytogenes count, a_w value, salt concentration and LAB growth in sausages during the ripening period of 21 days. The main parameters, maintained negative exponential growth rate of L. monocytogenes in fermented sausages, are a_w value decrease and LAB (starter culture), which stopped L. monocytogenes growth at the beginning of fermented sausage maturation. If fermentation process went technically and hygienically correctly, the fermented (cold-smoked) sausages could be one of the safest meat products, because in real practice a low level contamination has been seen. The remaining count of L. monocytogenes in fermented sausage depends on the possible initial contamination level and could exceed the European Union regulation value 2.0 lg (CFU g⁻¹) for ready-to-eat products when contamination at first is more than lg 5.0.

Keywords: Listeria monocytogenes, lactic acid bacteria, fermented sausages, water activity, pH.

Introduction
Listeria monocytogenes is an ubiquitous bacterial pathogen that can be found in a large number of food products and can survive and multiply at refrigeration temperature (Lunden et al., 2003). Processed meat products such as fermented sausages are a part of major products associated with listeriosis (Thevenot et al., 2005). L. monocytogenes infection has a high mortality rate – 20–30% (Farber, Peterkin, 1991). In the United States, a zero tolerance of L. monocytogenes in ready-to-eat foods has been prescribed for several years (Shank et al., 1996), but the European Union regulation exceeded concentration of L. monocytogenes in ready-to-eat food to 100 colony forming units (CFU) per gram (Anonymous, 2005). Glass, Doyle (1989) found out that L. monocytogenes decreasing level in fermented sausages and ham would be 1–2 lg (sausages) in 14 days and 2–3 lg (ham) in 28 days. Because lactic acid bacteria (LAB) can grow under the same storage conditions as Listeria spp. (Bērziņš et al., 2007), many studies have been conducted to investigate if these gram-positive organisms can provide adequate competition against the pathogenic organisms that are also present. The safety of fermented (cold smoked) sausages depends on the presence of factors such as concentration of sodium nitrite and salinity, relatively low water activity (a_w), low pH value, and application of probiotics (Lahti et al., 2001), like LAB used in fermented meat products (Bredholt et al., 2001). Liu et al. (2005) investigated that acid, alkali, and/or salt treatments, commonly used in food product processing, may not be sufficient to eliminate L. monocytogenes. Petran, Zottolla (1989) observed the growth of L. monocytogenes at the minimum a_w of 0.92. Below these minimum a_w levels, cell death is proportionate to water activity (Miller, 1992). According to literature sources available, some recent studies in food safety have investigated non-thermal processing of ready-to-eat food products, but there is little information about survival of L. monocytogenes found in different ripening stages of fermented sausages when main bacteria growth factors changed in time. Therefore, the aim of the study was to determine the survival limits of L. monocytogenes inoculated in manufactured fermented sausages depending on the LAB, water activity (a_w), and pH value changes in ripening time.

Materials and Methods
Individual pieces of raw sausages, before smoking at 28 °C, in initial weight mean value of 0.394 kg, were inoculated internally with a cocktail of local (wild) strains of L monocytogenes (serotypes 1/2a and 4b) originally isolated from surfaces and meat products of the mother factory (Bērziņš et al., 2007). The inoculated samples were labelled and subjected to smoking and maturating processes. All manipulations with samples were done in laboratory conditions (20 °C, 75–80% RH). The measurements and tests were done on 0, 1st, 3rd, 5th, 7th, 14th and 21st day of maturation. Three batches of fermented (cold smoked) sausages were investigated (a total of 60 samples) and the mean values of lg (CFU g⁻¹) were estimated between each other, and in addiction of pH, moisture content, and water activity (a_w) changes at ripening time.

L. monocytogenes strains were incubated in half-Fraser base medium for 18 h at 37 °C. The fresh concentrated culture of the selected strains was measured by optical densities (densitometer DEN-1B, UK) and prepared with sterile half Fraser broth (CM0895, SR0166E, Oxoid) to obtain approximately 8.0 lg (CFU mL⁻¹), and then samples of dry sausages inoculated portionally
(1 mL of inoculate in 100 g of sample) randomly leading to beginning concentration of 6.0 lg (CFU g⁻¹).

Ingredients of a 100 kg fermented sausage raw material were: pork – 30 kg, beef – 10 kg, bacon – 35 kg, structural emulsion – 25 kg. Salt and species summary was 3,25 kg and starter culture – 0.02 kg (‘Optistart Plus’, prepared by Raps GmbH and Co.KG, Germany). The smoking, fermentation, and ripening process were carried out in climatic chambers (models HR-6000 and HR-9000 ‘Sorgo’ Austria) at 28 °C with a relative humidity of 95% on first 3 days, down to 75% RH and 14–15 °C on 4th to 21st day.

The determination of L. monocytogenes count, CFU g⁻¹, was done according to Standard ISO 11290-2:1198 A:2005 ‘Microbiology of food and animal feeding stuffs. Horizontal method for the detection and enumeration of L. monocytogenes. Part 2: Enumeration method’. Each experimental batch was free of L. monocytogenes before culture inoculation, detected with standard method. The samples were analyzed by numbering L. monocytogenes on 0, 1st, 3rd, 5th, 7th, 14th and 21st days of maturation with the nine-tube most-probable-number (MPN) method. For analysis, 10 g of a carefully mixed fermented sausage sample were blended with 90 mL of sterile buffered peptone water in a laboratory blender (Stomacher 400, Interscience, France) for 1 min. Decimal dilutions were made to obtain samples of 1, 0.1, 0.01, 0.001, and 0.0001 g. To determine the MPN, three consecutive dilutions were used. Afterwards, 0.1 mL of each target dilution was spread on two LM-selective plates (PALCAM, Oxoid) and incubated for 48 h at 37 °C. For confirmation of L. monocytogenes, five typical colonies from two selective plates at each sampling time were streaked on sheep blood agar plates and incubated for 24 h at 37 °C. Catalase-positive, gram-positive rods, produced hemolysis on sheep blood agar (CAMP-test), were considered L. monocytogenes (McKellar, 1994). Total count (CFU g⁻¹) of L. monocytogenes in fermented sausage samples were calculated with classical formula given in Enumeration method standard.

The determination of LAB count, CFU g⁻¹, was done according to standard ISO 15214:1998 Microbiological methods of food and animal feeding stuffs - Horizontal method for the enumeration of mesophilic lactic acid bacteria - Colony-count technique at 30 °C.

pH was measured at the same time as other measurements. Three individual pieces of sausages were measured each time, and then mean pH value was calculated. The pH-meter Testo 205 (Testo AG Germany), with automatic temperature compensation, was applied. Meter calibration was done according to 2 point method with pH standard solutions 4.01 and 7.00. Water activity was measured with PawKit (Decagon) water activity meter. Calibration of device was done with saturated NaCl (sodium chloride) 6.0 molal standard solution (0.760 aW at 20°C). Samples for water activity measurement were collected in original polyethylene vessels with caps and measured immediately after collecting.

The digital salt-meter “PAL-ES2” was used for direct measurement of NaCl concentration in fermented sausages. The device have been calibrated with 2.50% NaCl solution AB250 from manufacturer (Atago, Inc. US) directly before measuring.

The determination of nitrite content in fermented sausages by ISO 2918:1975 standard was used for measuring nitrite on photometry method with 538 nm wavelength.

Statistical analysis. All experiments were reiterated three times, and tests were triplicated. The results represent the mean ± standard deviations (SD). Means were compared by Student’s t-test. Differences were considered statistically significant when p<0.05.

Statistical analysis was conducted with SPSS 17.0 (SPSS, Chicago, Ill., USA). Tables and chart figures were done by means of MS Excel 2007 appliances. To show the parameter changes in time, regression curves have been made for LAB, L. monocytogenes, aW, and pH. The main factors, affecting the bacterial growth, have been calculated by correlation (SPSS, Factor analysis).

Results and Discussion

The results of the physicochemical parameters and bacterial analysis of the fermented sausages at the beginning and the end of the ripening time are reported in Table 1. The values of pH were about 4.6 in the final product - typical of medium acidity sausages, and this was the result of the classical trend of microbial growth in the fermented sausages, where LAB are increasing in numbers at the very beginning of the fermentations (Figure 1), producing acids and a decrease in the pH, followed in the phases of maturation by the activity of micrococci that are able to neutralize the acids produced (Comi et al., 2005). The value of water activity (aW) showed a constant decrease during the maturation reaching final values of 0.80–0.82, and moisture of 251–258 g kg⁻¹. The final value of the salt content was around 40 g kg⁻¹, while the final nitrite about 9 mg kg⁻¹ of NO₂⁻. These changes of parameter were due to the effect of dehydration (Comi et al., 2005). Mean value of weight losses over 21 days of ripening, when relatively constant weight reached 75–76% of relative humidity of air (RH) in the climatic chamber, was approximately 110 and 117 g of samples with and without starter culture accordingly. Losses movement significantly (p<0.001) correlated with the mean value of moisture content.

Due to good adaptation of LAB to meat environment and their faster growth rates which were displayed during fermentation and ripening of sausages, they became the dominant microflora (Drosinos et al., 2005). The total count of LAB changes in fermented-cold-smoked sausages with and without starter culture is shown in Figure 1. Theoretically in sausage (A) by starter culture, calculated to 1 g sausage raw mass, lg 9.4 Lactobacillus sakei L110, lg 9.4 Staphylococcus xylosus, and lg 8.0 Debaryomyces hansenii were added.
The measured parameters values on start 0th day (S) and finishing 21st day (F)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Time</th>
<th>Start (S)</th>
<th>Finish (F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L.monocytogenes, lg (CFU⁻¹)</td>
<td>S</td>
<td>6.57±0.26</td>
<td>6.83±0.12</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>1.42±0.43</td>
<td>2.86±0.36</td>
</tr>
<tr>
<td>Total count of lactic bacteria, lg (CFU⁻¹)</td>
<td>S</td>
<td>5.72±0.18</td>
<td>3.35±0.15</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>9.41±0.32</td>
<td>6.95±0.27</td>
</tr>
<tr>
<td>Mean sausage weight, kg</td>
<td>S</td>
<td>0.39±0.05</td>
<td>0.39±0.05</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>0.28±0.05</td>
<td>0.28±0.05</td>
</tr>
<tr>
<td>Moisture content, %</td>
<td>S</td>
<td>34.78±0.40</td>
<td>39.43±0.38</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>25.12±0.38</td>
<td>25.88±0.36</td>
</tr>
<tr>
<td>pH value</td>
<td>S</td>
<td>5.80±0.02</td>
<td>5.76±0.016</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>4.67±0.016</td>
<td>4.59±0.015</td>
</tr>
<tr>
<td>aw value</td>
<td>S</td>
<td>0.96±0.002</td>
<td>0.95±0.002</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>0.82±0.003</td>
<td>0.80±0.003</td>
</tr>
<tr>
<td>Salt (NaCl) content, %</td>
<td>S</td>
<td>2.94±0.85</td>
<td>28.40±0.64</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>3.98±0.75</td>
<td>40.20±0.82</td>
</tr>
<tr>
<td>Nitrite (NO₂⁻) concentration, mg kg⁻¹</td>
<td>S</td>
<td>14.00±0.40</td>
<td>14.00±0.40</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>9.00±0.30</td>
<td>9.00±0.30</td>
</tr>
</tbody>
</table>

The difference of detected LAB count between sausage A and B variants on 0th day was approximately lg 2.37, that evident of artificially increasing count of LAB in sausage A by approximately 100 times in comparison to sausage B.

The most significant regression of bacteria count during all time investigated has been shown by a polynomial graph curve (Figure 1). In the bacterial growth period on first 7 days the best conformity (R²=0.98) to time, temperature, and interior factors are represented by linear regression in variant B (Figure 2).

It can be seen in Figure 1 and Figure 2 that exponential phase of lactic bacteria growth stops on the 7th day of ripening, when aw decreases below 0.92–0.90, and moves to stationary phase for next 7 days. The main species of LAB, detected before in the meat products prepared at the mother factory, and its minimal aw value by Vermeiren, Debevere (2004), were 0.94 for Lactobacillus brevis and 0.92 for Lactobacillus plantarum, which have been detected as the main LAB species in experimental sausage samples too. The main bacterial growth factor aw minimal values for starter culture components are: 0.91 L. sakei (Leroy, de Vuyst, 1999), 0.86 S. xylosus (Terra et al., 2007), and 0.81 D. hansenii (Aggarwal, Mondal, 2009). These different requests of minimal aw guaranty a constant level of pH during necessary ripening time.

Due to its water binding and ionic characteristics, salt affects the metabolism of a starter culture. The growth of lactic acid bacteria is sometimes enhanced in the presence of low content of sodium chloride (1 to 2%, 10–20 g kg⁻¹), but growth is clearly inhibited in the presence of NaCl content greater than 3% (30 g kg⁻¹) (Korkeala et al., 1992; Samapundo et al., 2010).

Figure 1. The polynomial changes of lactic acid bacteria count - lg (CFU⁻¹) values in fermented sausages with — (A) and without — (B) starter culture during ripening (p<0.01)

Figure 2. The linear regression trends of lactic acid bacteria count - lg (CFU⁻¹) values in fermented sausages with — (A) and without — (B) starter culture on first 7 days of ripening (p<0.05)

Figure 3. The polynomial regression trends of L. monocytogenes count - lg (CFU⁻¹) — (A) with and — (B) without starter culture in fermented sausages during ripening, ± SD values (p > 0.05)
Homofermentative LAB is more resistant to sodium chloride than heterofermentative LAB are, and strains resembling L. sakei have been shown to be more resistant than other strains. The initial L. monocytogenes inoculation concentration averaged 6.6–6.8 lg (CFU g⁻¹) was significantly (p<0.01) reduced at any ripening stage in both (A and B) sample variants (Figure 3). The sausage samples from this study had finally pH < 4.7 and a_w < 0.82. Such values guarantee no growth of L. monocytogenes (Vermeulen et al., 2007) and the rest count possibly depends on initial count. In both batches (A and B) the decrease of detected L. monocytogenes count showed a negative linear regression curve during the first 7 days (Figure 4) with lg (CFU g⁻¹) decreasing rate lg -0.54 (A) and lg -0.39 (B).

![Figure 4. The linear regression trends of L. monocytogenes count - lg (CFU g⁻¹) (A) with and (B) without starter culture in fermented sausages during the first 7 ripening days (p>0.05)](image)

However, it can be said that L. monocytogenes were inhibited and did not exceed the growth in all observed ripening time (21 day). As it can be seen in Table 1, and Figure 4, the addition of starter culture hastened L. monocytogenes live cells, and detected count of L. monocytogenes decreased two times. All changes of the physico-chemical parameters, except salt content, were decreased, but all of them did not support L. monocytogenes growth. All parameter changes more or less correlated (Table 2) between each other, but water activity is the parameter which summarizes these changes, and that is why it can be conferred as the main factor which limited pathogen growth in food products. The latest papers described that the growth of L. monocytogenes ceased at a cell concentration of about 10⁷ CFU mL⁻¹ when natural microflora of foods, such as lactic acid bacteria, entered stationary phase (Al-Zeyara et al., 2011).

pH, a_w, NaCl (g kg⁻¹), and NO₂⁻ (mg kg⁻¹) values changes are shown in double graph in Figures 5 and 6. The measurements of water activity show that L. monocytogenes growth would have been theoretically stopped on the 7th day of ripening when a_w decreased to 0.90 according to Vermeulen et al. (2007), but the observed results of bacterial count decrease made an idea of importance of a_w motion as the most significant factor against L. monocytogenes growing and survival in meat products.

![Figure 5. The changes of physico-chemical parameters: pH and a_w in fermented sausages with (A) and without (B) starter culture during 21 ripening days](image)

![Figure 6. The changes of physico-chemical parameters: NaCl and NO₂⁻ in fermented sausages with (A) and without (B) starter culture during 21 ripening days](image)

The samples of fermented sausages had a mean initial pH value of 5.80±0.02, which agrees with the results found by Paleari et al. (2003). A rapid decrease in pH was observed during the first three days of fermentation. The final pH of the fermented sausages had a mean value of 4.67–4.56±0.02; this drop in pH was due to lactic acid production by the starter culture used for fermentation (Vermeiren, Debevere, 2004). Lactobacilli are the major producers of lactic acid responsible for the decrease in pH and the increase in acidity during fermentation (Schillinger et al., 1991). Lactic and acetic acids are often suggested to be major contributors to the acid aromas and tastes and the

58
development of the texture of fermented sausage (Visessanguan et al., 2005).

Table 2

The correlation (r) values and their significance (p) levels between measured physic-chemical parameters and changes of inoculated *L. monocytogenes* count

<table>
<thead>
<tr>
<th>Parameters</th>
<th>A</th>
<th></th>
<th>B</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>Time (days)</td>
<td>-0.896</td>
<td>0.003</td>
<td>-0.963</td>
<td>0.000</td>
</tr>
<tr>
<td>Ig LAB</td>
<td>-0.865</td>
<td>0.006</td>
<td>-0.873</td>
<td>0.005</td>
</tr>
<tr>
<td>pH</td>
<td>0.609</td>
<td>0.073*</td>
<td>0.765</td>
<td>0.023*</td>
</tr>
<tr>
<td>$a_w$</td>
<td>0.867</td>
<td>0.006</td>
<td>0.980</td>
<td>0.000</td>
</tr>
<tr>
<td>NaCl, %</td>
<td>-0.954</td>
<td>0.000</td>
<td>-0.981</td>
<td>0.000</td>
</tr>
</tbody>
</table>

*Correlation is not significant

Under the fermentation and maturation conditions in this work, the decrease of *L. monocytogenes* count in fermented sausage was less intense than reported by other studies (Työppönen et al., 2003; Tolvanen et al., 2008) where an expressive decrease was observed at the beginning of ripening process. This is probably due to a lower pH and water activity in the first days of maturation noted in other studies, and higher initial *L. monocytogenes* concentration in our experiments.

The mean values of the decrease rate in *L. monocytogenes* count in batch B are bigger than those Glass, Doyle (1989) found in sausages without added lactic acid cultures.

No significant correlation was calculated in both experimental batches with and without starter culture between *L. monocytogenes* count and pH value. That could be explained by a relatively short time when pH value decreased to constant level and long stationary phase of pH value.

**Conclusions**

The main parameters, maintained negative exponential growth rate of *L. monocytogenes* in fermented sausages are $a_w$ value decrease and lactic acid bacteria, which stopped *L. monocytogenes* growing at the beginning of fermented sausage maturation. If fermentation process goes technically and hygienically correctly, the fermented (cold-smoked) sausages could be one of the safest meat products, because in real practice we observed a low level of contamination.

The remaining count of *L. monocytogenes* in fermented sausage depends on the possible initial contamination level and could exceed the European Union regulation value 2.0 lg (CFU g$^{-1}$) for ready-to-eat products when contamination at first is more than lg 5.0.

**References**


MICROBIOLOGICAL QUALITY OF MEAT PREPARATIONS AND MEAT PRODUCTS

Aija Melngaile, Elina Ciekure, Olga Valcina

Institute of Food Safety, Animal Health and Environment BIOR, Lejupes Street 3, Riga, Latvia, e-mail: aija.melngaile@bior.gov.lv

Abstract

The aim of the research was to perform trend analysis to reveal probable gaps and shortcomings in monitoring of microbiological contamination of meat preparations and meat products produced in Latvia to highlight proposals for further improvements. The results on microbiological contamination of foodstuffs obtained in the frame of producers’ self-control within HACCP procedures were used in the research. In total, 13 food types, including minced meat, breaded pork chop, different types of sausages, smoked meat products, aspic and liver paste were investigated. Using single factor analysis of variance (ANOVA) significant differences were revealed between food types, as well as large fluctuations in aerobic plate counts (APC) were demonstrated. According to the findings of the research, APC was significantly different \((p=0.001)\) for sausages. The maximum mean APC \((6.68±6.36 \text{ lg CFU g}^{-1})\) was found for liver sausage. APC was largely variable for smoked meat products. Significant difference \((p=0.01)\) was revealed with regard to APC for aspics tested one and six days after production. Hygiene indicator organisms \((Escherichia coli)\) and pathogens \((e.g. Salmonella spp.)\) most often were detected in meat preparations. Presence of coliforms was detected in aspics and liver paste. The results of the research suggest that development and usage of guidelines of good manufacturing practice for purposeful monitoring of microbiological contamination risk of meat preparations and meat products is relevant to ensure high level of protection of consumers’ health.

Keywords: microbiological contamination, meat preparations and products, good manufacturing practice.

Introduction

The safety of food must be assured by a preventative approach based on the application of a Hazard Analysis Critical Control Point (HACCP) at all stages of food chain. The HACCP system is a structured approach for identifying hazards and defining and implementing systems of adequate control. Risk-based programmes have been proved successful in achieving hazard control to the extent required for consumer protection. Microbiological examination of food and environmental samples is generally recommended to validate and verify the efficiency of foods safety and quality control (ICMSF, 2011; IFST, 1997). Foodborne disease or microbiological spoilage of food can result from the failure or inability to control microorganisms at one or more stages of food production. Therefore, the microbiological testing at various stages of food production is relevant to reveal and understand the characteristic trends in distribution of microbiological contamination (ICMSF, 2011; Schaffner, 2007; Burlingame and Pino, 2007; IFST, 1997).

It should be mentioned that only one official regulation concerning the microbiological quality of meat preparations is stated in food safety legislation, namely, salmonella spp. contamination must not be found in 10 g of minced meat and meat preparations intended for use after thermal processing. As regards ready-to-eat meat preparations, no legal requirement for microbiological safety is stated in the legislation (Commission Regulation 2073/2005).

To monitor microbiological quality of meat preparations and meat products guidelines and recommendations of international and national level have been developed in addition to legal requirements. According to the guidelines of good manufacturing practice the level of total microbiological contamination of raw meat and raw meat preparations should not exceed \(10^4\) (maximum \(10^7\) ) CFU g\(^{-1}\) and of thermally processed meat products – \(10^4\) CFU g\(^{-1}\). Escherichia coli contamination in raw meat and raw meat preparations should not exceed \(10^7\) (maximum \(10^9\) ) CFU g\(^{-1}\) and Enterobacteriaceae and Escherichia coli contamination in meat products – \(10^4\) (maximum \(10^6\)) and 10 (maximum \(10^3\) ) CFU g\(^{-1}\), respectively (ICMSF, 2011; IFST, 1997).

It is commonly suggested that microorganisms can enter meat preparation like sausages from meat, spices, and other ingredients, as well as from processing environment, equipment, and handlers that can have a significant impact on the microbiological status of the end-products. In general, heating during technological processing of meat products is an effective tool to reduce microbial counts of end-products (Güngör, 2010).

Development of preventive food safety assurance systems comprises both the identification of important food safety hazards and the introduction of regular monitoring measures in critical control points of technological processes. It is widely recognised that management of technological processes should be based on detailed analysis of product characteristics and process conditions to assess the potential impact on quality and safety of the ready-to-eat foods (Schaffner, 2007; Burlingame, Pino, 2007).

The aim of the research was to perform trend analysis to reveal probable gaps and shortcomings in monitoring of microbiological contamination of meat preparations and meat products produced in Latvia to highlight proposals for further improvements.

Materials and Methods

The data on microbiological contamination of food products obtained in frame of producers’ self-control within HACCP procedures (December 2012 – January 2014) were used to analyse microbiological quality of...
meat preparations and meat products produced in Latvia. Food samples were taken at three meat processing companies and were stored at temperature +4 °C after sampling and during delivering to the laboratory. All samples of meat preparations were taken one day after production, but samples of meat products were taken on different days of shelf-life (namely, one to 11 days after production) to evaluate distribution of microbiological contamination.

During the research food products were divided into two compatibility groups: meat preparations (no thermal processing used during production) and meat products (thermally processed foodstuffs). In total 144 samples of meat preparations and 141 samples of meat products were analysed for the aerobic plate count (APC). 724 samples of meat preparations and products were analysed for presence of hygiene indicator organisms (coliforms and *Escherichia coli*) and pathogens (*Salmonella* spp., *Listeria monocytogenes*, *Staphylococcus aureus*, and sulfite – reducing clostridia).

To perform the mathematical analysis of the analytical results, food products of the two compatibility groups were grouped into 13 compatibility types on the base of characteristic ingredients and/or production technology, namely: minced meat (110 samples, including pork, beef and mixed), hot-smoked meat products (33 samples), frankfurters and small sausages (33 samples), cooked sausages (29 samples), breaded pork chops (24 samples), aspics (19 samples), kebab or minced pork skewers with added spices (10 samples), semi-dried sausages (10 samples), hot-smoked sausages (six samples) liver sausages (four samples), semi-smoked sausages (four samples), liver pate (two samples), and cold-smoked sausages (one sample). Following standards were used for testing of microbiological quality of meat preparations and products: standard LVS EN ISO 4833:1:2014 was used for detection of Aerobic plate counts (APC), standard LVS EN ISO 11290–2:1998/A1:2005 – for testing of *Listeria monocytogenes*, standard GOST R 52815-2007 – for detection of *Staphylococcus aureus*, standard LVS EN ISO 6579:2003 amended by standard LVS EN ISO 6579:2003/AC:2006 – for testing of *Salmonella* spp., standard GOST 29185-91 – for testing of presence of sulphite-reducing clostridia, standard LVS ISO 16649-2:2007 – for detection of presence of *Escherichia coli*, and standard GOST R 52816-2007 – for detection of coliforms in food samples.

The statistical analysis of the analytical data was performed using single factor analysis of variance (ANOVA). Results on microbiological testing of cold-smoked sausage were excluded from further statistical analysis because only one sample was tested. In cases when p-value was p=0.05, it was considered that features under research are mutually dependent with probability of 95%. The data of mathematical analysis were described with help of histograms.

Results and Discussion

The results of the mathematical analysis indicate that APC values for meat products and meat preparations are not significantly different (p=0.23) (Fig. 1 and Fig. 2).

![Figure 1. Minimum, maximum and median of APC levels in meat preparations (lg CFU g⁻¹)](image)

The findings of the research suggest that meat products, which are subjected to thermal processing during the production process, may still contain high numbers of microorganisms.

The results of the mathematical analysis indicate that the value of APC within the group of meat preparations (foodstuffs that have not undergone thermal processing) is substantially different (p=0.02) and dependent on the method of technological treatment. The highest maximum APC value (10.43±9.47 lg CFU g⁻¹) and the highest mean APC value (9.14±9.47 lg CFU g⁻¹) was found for samples of breaded pork chops (Fig. 1). Significant difference was not revealed for APC in kebab with added paprika and kebab with added herbs (p=0.15). Despite the fact that samples of meat preparations were taken and analysed only one day after production a huge variation between APC values was observed. Standard deviation of APC for breaded pork chops and minced meat was even higher than the mean value of APC (Table 1).

Standard deviation is usually used to describe the distribution in relation to the mean value. Basically, a large standard deviation means that the values in a statistical data set are farther away from the mean, on average. Thus, from mathematical point of view, a large standard deviation found in frame of the research reflects a large variation between APC values (or the existence of data with extreme values) for the types of meat preparations that were studied. From food safety viewpoint, the results that deviate significantly from the trend may indicate a tendency towards a situation which is out of control and may highlight the need for attention before control is lost. It is always very essential to understand the nature of potential hazards that may be presented by raw materials (ICMSF, 2011; Schaffner, 2007; IFST, 1997).
The lowest minimum, minimum and mean APC values were found for minced meat samples. The mean value of APC for raw meat (5.08±10^3 ± 5.16x10^3 CFU g^-1) is still in line with international guidelines of good manufacturing practice and Finnish recommendations to good quality of minced meat, however, the huge standard deviation suggests the large variability of microbiological quality of minced meat produced in Latvia. The maximum and mean APC values for breaded pork chops are well above the value recommended in the international guidelines (10^2 CFU g^-1) (ICMSF, 2011; IFST, 1997; Skrøkkø, 1997). The results of the mathematical analysis indicate that the value of APC within the group of meat products (foodstuffs that have been thermally processed) is substantially different (p=0.0005) and dependent on the method of technological processing. As aspic samples were analysed on several days after production – one, two or six days after production – the APC values for aspic were excluded from the mathematical analysis. The findings of the research suggest that microbiological quality of ready-to-eat meat products should be improved. The maximum and mean values of APC for liver sausages and semi-smoked sausages, as well as maximum APC values for frankfurters, small sausages and cooked sausages are not in line with international guidelines of good manufacturing practice (10^2 CFU g^-1) and are close to the maximum acceptable level (10^7 CFU g^-1) (ICMSF, 2011; IFST, 1997). The highest maximum APC (6.68±6.36 lg CFU g^-1) and the highest mean APC (6.16±6.36 lg CFU g^-1) was found for samples of liver sausage (Table 1, Fig. 2). High maximum and mean values of APC were also revealed for semi-smoked sausages and smoked meat products.

A huge variation between APC values was observed. Standard deviation of the mean values of APC for many meat products was much higher than the mean value of APC (Table 1). Like in case with meat preparations, the large standard deviations indicate that the APC values are largely dispersed and extreme values are farther away from the mean value. Thus, the large standard deviations found in frame of the research reflect a large variability of APC values within types of meat products that were studied. However, from food safety point of view the results that deviate significantly from the common trend may indicate a tendency towards a situation which is out of control and may highlight the need for attention before control is lost (ICMSF, 2011; Schaffner, 2007; IFST, 1997). Comparatively less variations in APC values were revealed for hot-smoked sausages and semi-dried sausages. The results of the mathematical analysis indicate that the value of APC for aspic analysed one, two and six days after production is substantially different (p=0.01). In addition, the correlation coefficient r²=0.75 indicates a strong positive correlation between level of APC and day of storage (Fig. 3). It is necessary to emphasize that the mean value of APC for aspic analysed six days after production (8.33±8.40 lg CFU g^-1) was about 300 times higher than for aspic analysed only one day after production (4.56±4.73 lg CFU g^-1). Maximum APC value that was observed for aspic tested six days after production was the highest one within the group of meat products (8.70±8.40 lg CFU g^-1).

**Table 1**

<table>
<thead>
<tr>
<th>Type of meat preparation or product</th>
<th>Mean value</th>
<th>Standard deviation</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minced meat</td>
<td>5.08</td>
<td>5.16</td>
<td>4.83</td>
</tr>
<tr>
<td>Kebab</td>
<td>7.64</td>
<td>7.48</td>
<td>7.67</td>
</tr>
<tr>
<td>Breaded pork chops</td>
<td>9.14</td>
<td>9.74</td>
<td>7.86</td>
</tr>
<tr>
<td>Cold-smoked sausages</td>
<td>1.00</td>
<td>-</td>
<td>1.00</td>
</tr>
<tr>
<td>Semi-dried sausages</td>
<td>3.01</td>
<td>2.85</td>
<td>3.10</td>
</tr>
<tr>
<td>Liver pate</td>
<td>3.51</td>
<td>1.85</td>
<td>3.51</td>
</tr>
<tr>
<td>Hot-smoked sausages</td>
<td>3.99</td>
<td>3.73</td>
<td>4.08</td>
</tr>
<tr>
<td>Cooked sausages</td>
<td>4.86</td>
<td>5.52</td>
<td>2.04</td>
</tr>
<tr>
<td>Frankfurters, small sausages</td>
<td>4.91</td>
<td>5.58</td>
<td>2.90</td>
</tr>
<tr>
<td>Smoked meat products</td>
<td>4.94</td>
<td>5.55</td>
<td>2.56</td>
</tr>
<tr>
<td>Semi-smoked sausages</td>
<td>5.12</td>
<td>5.41</td>
<td>3.51</td>
</tr>
<tr>
<td>Liver sausage</td>
<td>6.16</td>
<td>6.36</td>
<td>5.69</td>
</tr>
<tr>
<td>Aspic, day 1</td>
<td>4.56</td>
<td>4.73</td>
<td>4.19</td>
</tr>
<tr>
<td>Aspic, day 2</td>
<td>5.85</td>
<td>6.08</td>
<td>3.23</td>
</tr>
<tr>
<td>Aspic, day 6</td>
<td>8.33</td>
<td>8.40</td>
<td>8.24</td>
</tr>
</tbody>
</table>

The findings of the research suggest that the APC values are largely dispersed and extreme values are farther away from the mean value. Thus, the large standard deviations found in frame of the research reflect a large variability of APC values within types of meat products that were studied. However, from food safety point of view the results that deviate significantly from the common trend may indicate a tendency towards a situation which is out of control and may highlight the need for attention before control is lost (ICMSF, 2011; Schaffner, 2007; IFST, 1997). Comparatively less variations in APC values were revealed for hot-smoked sausages and semi-dried sausages.

The results of the mathematical analysis indicate that the value of APC for aspic analysed one, two and six days after production is substantially different (p=0.01). In addition, the correlation coefficient r²=0.75 indicates a strong positive correlation between level of APC and day of storage (Fig. 3). It is necessary to emphasize that the mean value of APC for aspic analysed six days after production (8.33±8.40 lg CFU g^-1) was about 300 times higher than for aspic analysed only one day after production (4.56±4.73 lg CFU g^-1). Maximum APC value that was observed for aspic tested six days after production was the highest one within the group of meat products (8.70±8.40 lg CFU g^-1).

**Figure 2.** Minimum, maximum and median of APC levels in meat products (lg CFU g^-1)


The statistically significant difference (p=0.001) was found with regard to APC for different types of sausages. The minimum APC for the most types of sausages was <1 lg CFU g^-1 with the exception of liver sausages and hot-smoked sausages, the minimum APC of which was 3.43±6.36 lg CFU g^-1 and 3.32±3.73 lg CFU g^-1, respectively (Table 1, Fig. 2).
The maximum APC value (6.68±6.36 lg CFU g⁻¹) was found for liver sausage.

Figure 3. APC level in aspics lg CFU g⁻¹, referring to different days of storage

Day1 – one day after production, Day 2 – two days after production, Day 6 – six days after production

The research revealed a huge fluctuation between APC values for liver sausages produced in different factories, namely the APC values for liver sausage tested on the day of production (5.99 lg CFU g⁻¹) and for liver sausage tested two days after production (6.68 lg CFU g⁻¹) were much higher than for liver sausage the sample of which was analysed 10 days after production (3.43 lg CFU g⁻¹). Unlike for liver sausages, comparatively low APC was revealed for liver pate (3.52 lg CFU g⁻¹) even 11 days after production that may suggest more use of food preservatives.

Although in general it could be expected that APC for cooked meat products should be higher than for dried meat products, no significant difference was detected with regard to APC for smoked sausages and cooked sausages (p=0.55). The findings may suggest about inadequate parameters of technological processing or cross-contamination of smoked sausages after production.

Like for liver sausages a huge variation in APC values was found for smoked pork products produced in different factories; for example, APC varied greatly from <1 lg CFU g⁻¹ to 6.26 lg CFU g⁻¹ on the day of production of smoked pork products, as well as for sample tested on the day of production the APC value was much higher (6.26 lg CFU g⁻¹) than for several other samples tested 5 days after production (<1 lg CFU g⁻¹).

Hygiene indicatororganisms and pathogens were detected in 72 (10%) of 724 meat product samples tested.

In total, coliforms were tested in 77 samples of meat products, and only two samples – samples of aspic and liver pate – were positive. The presence of *Escherichia coli* (≥1 lg CFU g⁻¹) was detected in 28% of 188 samples tested. *Escherichia coli* were not found in thermally processed meat products. Most often *Escherichia coli* were detected in kebab samples, namely it was detected in 19 of 20 kebab samples tested. *Escherichia coli* were detected in 31 minced meat samples (in 23% samples of 135 samples tested). Statistically significant difference (p=0.008) was revealed in relation to *Escherichia coli* count for minced meat (mean value 2.04±1.94 lg CFU g⁻¹) and for kebab (mean value 3.11±3.40 lg CFU g⁻¹). The average *Escherichia coli* count in breaded pork chop was 1.70±1.45 lg CFU g⁻¹. The results indicate that *Escherichia coli* count in kebab exceed 10² CFU g⁻¹ that is internationally recommended reference value of good hygiene practice (ICMSF, 2011; IFST, 1997).

Pathogenic microorganisms were mainly found in raw meat preparations. *Salmonella* spp. were detected in 8 minced meat samples of 282 samples of meat preparations and meat products tested. According to the results of *Salmonella* serotyping, *Salmonella typhimurium* were revealed in five tests. *Listeria monocytogenes* were detected in 4 minced meat samples of 110 samples of meat preparations and meat products that were tested for the presence of *Listeria monocytogenes*.

Presence of *Staphylococcus aureus* was not detected in 29 samples of sausages and smoked meat. Sulphite – reducing clostridia were found in one sample of semi-dried sausage.

It should be taken into account that the potential for growth and/or toxin production of residual microbial population in the end-products depends on the types of organisms present in food and their ability to grow to a level of concern under the storage conditions applied during the shelf life (ICMSF, 2011; Schaffner, 2007; IFST, 1997). Therefore, the microbiological quality of raw meat and raw meat preparations, as well as methods of technological processing of meat and meat preparations is of great importance to control the microbiological contamination of ready-to-eat meat products.

The findings of the research demonstrate characteristic trends in prevalence of microbiological contamination in meat preparations and meat products produced in Latvia, including:

- high variabilty of microbiological quality of meat preparations and meat products was revealed during shelf-life of foodstuffs, suggesting that foods of significantly different microbiological quality are marketed;
- high numbers of microorganisms in thermally unprocessed meat preparations, especially in breaded pork chops were demonstrated, indicating that maximum and mean APC values can be well above the international guidelines of good manufacturing practice;
- also, high numbers of microorganisms in certain thermally processed meat products were detected, indicating that maximum and mean values of APC for liver sausages, semi-smoked sausages, as well as for frankfurters, small sausages and cooked sausages are not in line with international guidelines of good manufacturing practice;
- microbiological contamination of meat products was generally not dependent on the method of
technological processing, demonstrating that high risk products can be found within different meat products types – for example, statistically significant difference was not found between APC values of smoked and cooked sausages:

- the mean value of APC for aspics increased dramatically within six days after production, proving that setting of adequate “use by” is not carefully investigated;
- pathogenic and indicatororganisms were found in raw meat preparations, indicating that raw meat and raw meat preparations can be a source of pathogens and further thermal processing is therefore of great importance, including giving precise cooking instructions to final consumers on labels;
- the presence of coliforms was detected in aspics and liver pate, suggesting that occasionally presence of pathogens can not be excluded.

The most probable reasons of high microbial counts in meat preparations and meat products might be poor hygienic quality of raw meat and other ingredients, inadequate storage and thawing conditions, as well as contamination from technological equipment and via hands of personnel. In addition, the inadequate temperature-time parameters of thermal processing, cross-contamination of ready-to-eat products after production process or due to contaminated packaging material can enhance the microbial load of end-products.

A clear understanding of the effects of food handling practices and processing technologies on microorganisms and, in particular, on pathogens in foods, including their survival and growth characteristics is essential (ICMSF, 2011; Schaffner, 2007; IFST, 1997). The results of the research suggest that purposeful investigation of the microbiological quality of meat preparations and meat products with the aim to perform trend analysis of microbiological contamination should be carried out at the sites of production. In order to reduce presence, survival and multiplication of pathogens in end-products, verification of good manufacturing practice and monitoring procedures, as well as validation of overall HACCP procedure is of great importance.

Conclusions
A huge load of total microbiological contamination in meat preparations and meat products can lead to introduction of pathogenic microorganisms in foods therefore risk assessment on the base of trend analysis is relevant.

To conclude on bottle-necks during production of meat preparations and products, purposeful testing of indicatororganisms can be recommended. The analysis of trends in microbiological contamination of food and environmental samples should be used to improve theoretically developed HACCP plans at the level of individual food production companies. In addition, quality of marketing conditions should be studied by producers for better understanding of changes in microbiological quality of foods during shelf-life. The results of the research suggest that development of guidelines on good manufacturing practice to explain common principles of trend analysis and purposeful monitoring of microbiological contamination of meat preparations and meat products is essential to ensure continuous improvement of microbiological quality of foods and thereby a high level of protection of consumers’ health.

Acknowledgment
This study was made possible through the kind contribution and support of the Latvian Federation of Food Enterprises.

References
THE INFLUENCE OF ENVIRONMENTAL CONDITIONS ON WINTER WHEAT WHOLEMEAL PROTEIN CONTENT AND RHEOLOGICAL PROPERTIES

Anda Linina\textsuperscript{1}, Antons Ruza\textsuperscript{1}, Daiga Kunkulberga\textsuperscript{2}, Tatjana Rakcejeva\textsuperscript{2}

\textsuperscript{1}Institute of Agrobiotechnology, Faculty of Agriculture, Latvia University of Agriculture, Liela street 2, Jelgava, Latvia, e-mail: anda.linina@llu.lv
\textsuperscript{2}Faculty of Food Technology, Latvia University of Agriculture, Liela street 2, Jelgava, Latvia

Abstract

The aim of this investigation was to clarify variation of protein content on winter wheat (\textit{Triticum aestivum} L.) grain, water absorption and mixing properties of wholemeal dough, depending on harvest year weather conditions (2010–2012) and cultivar. Trial included winter wheat cultivars ‘Bussard’ and ‘Zentos’. The farinograph water absorption (WA) and wholemeal dough mixing characteristics – dough development time (DDT), dough stability time (ST) and degree of softening (DS12) were tested by Brabender Farinograph (ICC 115/1). The data show that experimental year and cultivar significantly (p<0.05) affected protein content, farinograph water absorption and mixing properties of winter wheat wholemeal dough. The influence of year was confirmed on higher level for grain protein content and wholemeal dough stability time, compared with the cultivar effect. Cultivar had a much stronger effect on wholemeal dough water absorption, dough development time and degree of softening than year. Cultivar ‘Bussard’ wholemeal had higher protein content, water absorption, longer stability time and shorter degree of softening compared with ‘Zentos’. The results demonstrate that the quality of the studied varieties meets the requirements for high-grade wheat for food consumption, and are suitable for wholegrain flour production and baking. The positive correlation (r=0.972) existed between protein content and dough stability. Protein content correlated negative (r=−0.893) with dough degree of softening. Dough stability time had negative correlation (r=−0.878) with degree of softening.

Keywords: winter wheat, protein, rheological properties, Farinograph.

Introduction

Wheat (\textit{Triticum aestivum} L.) is a dominant cereal crop in the Latvia, and more and more is used in the preparation of bread made from whole wheat grain flour. Whole grain foods are an important part of the diet because they provide many nutrients (Kunkulberga et al., 2007b). The wheat kernels contain a germ, pericarp layers (outer and inner), seed coat, aleurone layer and starchy endosperm. The objective of milling is to separate the starchy endosperm from the kernel, and to ground it into flour. The aleurone layer, pericarp layer and seed coat form the bran. When white flour is produced, many important nutrients including dietary fibres are removed, because these components are mainly located in bran and germ (Dewettinck et al., 2008).

Quality parameters of winter wheat are not stable between production years because of the inconsistency of the variables, such as initiation of the growing season, distribution of rainfall and heat units available for crop growth during corresponding phases of plant growth and development (Linina, Ruza, 2004).

Protein content is commonly used as predictor of baking quality (Koppel, Ingver, 2010). The temperatures and water stress occurring during grain filling period affects changes in wheat protein aggregation (Daniel, Trboi, 2002). Grain protein content significantly varied depending on the differences among cultivars (Linina, Ruza, 2012). During ripening wheat needs sunny and warm weather and moderate moisture. These conditions secure biological maturity and acceptable technological and rheological properties of grain (Krejčirova et al., 2006).

Evaluation of rheological properties of flour by farinograph is popular among the millers, bakers, grain handlers and wheat breeders. The rheological characteristics reflect the dough properties during processing and the quality of the final product. Strong flours are characterized by a long dough development time, high stability with a small degree of softening, while poor flours weaken quickly, resulting in high degree of softening (Shahzadi et al., 2005). Rheological properties of wheat mainly depend from cultivars diversity and the growth conditions. Panozzo (2000) reported that year meteorological conditions and cultivar interactions were significant for the dough rheological characteristics.

In Latvia there were no made investigations about winter wheat wholemeal rheological properties depending on weather conditions and cultivar till now. Therefore, the aim of the present research was to investigate the influence of weather conditions in three harvesting years and two different cultivars on the content of wholemeal protein, and the farinograph water absorption, dough development time, stability time and degree of softening.

Materials and Methods

\textbf{Study fields}. Field experiments in years 2010, 2011 and 2012 were conducted at the Latvia University of Agriculture, Study and Research farm “Peterlauki” on silt loam brown lessive soil with close to neutral acidity (pH\textsubscript{K\textsc{cl}} 6.9), medium high phosphorus and potassium, humus content 2.7 g kg\textsuperscript{−1}. Registered winter wheat (\textit{Triticum aestivum} L.) bread cultivars from Germany ‘Bussard’ and ‘Zentos’ were sown after black fallow. Both cultivars are with high bread – making quality. Phosphorus and potassium fertilizers were applied in
Weather data collection. The air temperature in investigation years (Table 1) in April was by 0.8–2.5 °C higher compared with long-term average observations; also May was by 0.3–1.3 °C warmer, which promoted plant growth and development. Average daily temperature in June 2010 and 2011 was warmer by 0.9–2.0 °C which contributed to the accumulation of protein. In 2012 air temperature was lower than 1.1 °C, compared to long-term average data. Temperature in the grain filling period (July), which is most decisive for grain quality formation, was in 2010 by 4.4 °C warmer and by 2.7 °C warmer in 2011, while in 2012 only by 1.2 °C higher than the long-term average mean data.

Table 1

<table>
<thead>
<tr>
<th>Month</th>
<th>Average temperature °C</th>
<th>2010</th>
<th>2011</th>
<th>2012</th>
<th>LTM*</th>
</tr>
</thead>
<tbody>
<tr>
<td>April</td>
<td>6.2</td>
<td>7.9</td>
<td>6.4</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td>May</td>
<td>12.6</td>
<td>11.6</td>
<td>12.2</td>
<td>11.3</td>
<td></td>
</tr>
<tr>
<td>June</td>
<td>16.1</td>
<td>17.2</td>
<td>14.1</td>
<td>15.2</td>
<td></td>
</tr>
<tr>
<td>July</td>
<td>21.2</td>
<td>19.5</td>
<td>18.0</td>
<td>16.8</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>14.0</td>
<td>14.1</td>
<td>12.7</td>
<td>12.2</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sum of precipitation, mm</th>
<th>April</th>
<th>May</th>
<th>June</th>
<th>July</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>48</td>
<td>85</td>
<td>60</td>
<td>298</td>
<td>122</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>56</td>
<td>78</td>
<td>179</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>106</td>
<td>45</td>
<td>95</td>
<td>197</td>
<td>111</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>51</td>
<td>75</td>
<td>82</td>
<td>62</td>
</tr>
</tbody>
</table>

*S–LTM—long-term mean

Water availability has effect on wheat grain quality. Precipitation in April 2010 and 2011 was close to long-term average, but in 2012 by 265% more than long-term means data.

May in 2010 was wet, when precipitation was 164% higher than the long-term average for this month, in 2011 and 2012 precipitation was close long-term mean data for this month.

Precipitation in June 2010 and 2011 was close to long-term mean; but in 2012 by 126% more than long-term means data. July in 2010, 2011 and 2012 was very rainy, respectively by 365%, 219% and 241% exceeded the long-term average data.

Technological properties of wheat. The protein content and rheological properties of wholemeal wheat were determined at the Latvia University of Agriculture, in the Grain and Seed Research Laboratory and Laboratory of Food analysis. Grains were milled to wholemeal using Laboratory Mill 3100 (Perten Instruments, Sweden) at a particle size of 0.8 mm. Wheat technological properties were analyzed in duplicate. Grain protein content (PC) was calculated multiplying total nitrogen content by factor 5.7 determined by Kjeldahl method (ICC 105/2; Kjeltc system 1002, Foss Tecator AB, Sweden). The farinograph water absorption (WA, 14%) and dough mixing characteristics—dough development time (DDT), dough stability time (ST) and degree of softening (DS12) were tested by Brabender Farinograph with a mixer using 300 g of flour, with slow blade rotation speed 63 rpm and measurement control system software 2.5.17 (Brabender, Germany; ICC 115/1).

Statistical analysis. Experimental data evaluation was done using two – factor analysis of variance by Fisher’s criteria and least significant difference (LSD0.05) were applied to estimate the effects of year (weather conditions) and cultivars. Component of variance ANOVA for each quality characteristic were expressed as percentage to illustrate the relative impact of each source to the total variance. Correlation analysis between protein content and wholemeal rheological properties was also carried out.

Results and Discussion

Protein content (PC) is one of more important indicators of grain quality for the bread making industry (Ruzgas, Liutukas, 2008). Grains with protein content 12–13% are suitable for bread making; however, grains with higher protein content traditionally are used for improving the properties of lower quality grains. Grain processing companies in Latvia are limited requirements for A quality class wheat is protein contents >145 g kg⁻¹, first class > 140 g kg⁻¹, second class >130 g kg⁻¹ and third class – > 120 g kg⁻¹.

Average data in our experiment (3 years) suggest (Table 2) that protein content in cultivar ‘Bussard’ grains was 143.4 g kg⁻¹, it was statistically significantly higher compared to ‘Zentos’ (122.6 g kg⁻¹). Grain protein content significantly varied depending on the cultivars and meteorological conditions (Ruza et al., 2002; Kunkulberga et al., 2007a; Skudra, Linina, 2011).
April to July during the years 2010 and 2011 exceeded 14 °C and this was favourable for protein synthesis, therefore, in cultivar ‘Bussard’ grain average protein content was 153.6 g kg⁻¹, while in ‘Zentos’–132.7 g kg⁻¹. Similar scientific results were obtained in the trial Dotnuva (Lithuania) Cesevičiene and co-authors (2009), they conclude, that warmer weather with more sunny days is more favourable for the accumulation of protein content in wheat grains. The lowest protein content was identified in both analysed cultivars in year 2012. Grain quality mainly was affected by the weather conditions during the ripening period (Cesevičiene, 2012). Cool (average air temperature in growing season 12.7 °C) and rainy weather in 2012 during grain filling – ripening stage was adverse for protein accumulation. In cultivar ‘Bussard’ grain average protein content was 124.9 g kg⁻¹ and corresponded to third class, especially low protein content accumulated in the cultivar ‘Zentos’ grain – 102.5 g kg⁻¹.

In our experiment, according to Fisher’s criteria, the weather conditions and cultivar had a significant (p<0.05) impact on the grain protein content. Grain protein content did not significantly depend either on the year–cultivar interaction. Similarly, the analysis of variance for two cultivars and 3 experiment years suggest that winter wheat grain protein content by 63.3% depended on weather conditions (year), but the influence cultivar was also remarkable – 36.4% (Table 3). Influence of the year was most remarkable also in the investigation with 15 winter and 14 spring wheat cultivars in the years 2004–2007 in Estonia (Koppel, Ingver, 2008).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>PC</th>
<th>WA</th>
<th>DDT</th>
<th>ST</th>
<th>DS12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>63.3</td>
<td>28.1</td>
<td>37.4</td>
<td>59.1</td>
<td>28.1</td>
</tr>
<tr>
<td>Cultivar</td>
<td>36.4</td>
<td>66.4</td>
<td>51.8</td>
<td>37.8</td>
<td>68.9</td>
</tr>
<tr>
<td>Year/cultivarinteraction</td>
<td>0.2 ns</td>
<td>3.1 ns</td>
<td>10.6</td>
<td>3.0</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Shattering, WA significantly (Table 3). WA was mostly affected by the cultivar (66.4%) however; the year factor was also remarkable (28.1%). In Koppel and Ingver (2010) experiments with 11 winter wheat cultivars in the years 2005-2009. WA dependency on year complete to 63.6% but the influence of cultivar – 24.3%. Dough development time (DDT) indicates the relative strength of the wheat flour and can also reflect the level of water absorption in the test. Therefore, if the dough development time is shorter, the less time is needed to mix the dough (Sabovics, Straumite, 2012). In the present experiments, the dough development time of both analyzed cultivars was high (Table 5).

A dough development time of wholemeal from ‘Bussard’ was 4.75 min in average, and this is by 1.11 min lower compared with ‘Zentos’ (5.86 min). Sabovics and Straumite (2012) found similar results in Latvia: they tested wholemeal triticale properties by farinograph and determined dough development time as 5.95 min, while Mašauskiene and Cesevičiene (2006) found that dough development time for cultivar ‘Zentos’ white flour was 2.25–3.43 min.
Prabhasankar et al., (2002) and Shahzadi et al., (2005) found that wholemeal dough require a longer development time compared to white flour because it contains bran. The weather conditions in the investigations years, cultivars and year–cultivar interaction had a significant (p<0.05) impact on the wholemeal DDT. DDT was more affected by the cultivar (51.8%) than by the year (37.4%) and lowest impact belonged to interaction cultivar-year (10.6%).

*Dough Stability (ST) indicates* the time when the dough maintains maximum consistency and a good indication of dough strength (Karaoglu, 2011). ST is measure that is expected by baking industry for producing yeast bread. Good quality dough could be stable for 4–12 min. Satisfactory ST is about 6 min. Average dough stability (Table 6) of cultivar ‘Bussard’ was 9.01 min, while it for ‘Zentos’ was 6.77. The dough stability should relate with the cultivars peculiarities mainly (Cesevičiene et. al., 2012).

**Table 5**

<table>
<thead>
<tr>
<th>Cultivar, Year, factor B</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bussard</td>
<td>2010 4.55</td>
</tr>
<tr>
<td>Zentos</td>
<td>5.99</td>
</tr>
<tr>
<td>Average B</td>
<td>5.77</td>
</tr>
</tbody>
</table>

**Degree of softening (DS12)** is the difference between the centre of the curve at the end of the dough development time and the centre of the curve 12 minutes after this point. Dough mixing qualities are considered satisfactory when the degree of softening is below 70 FU. When this value exceeds 110 FU, the dough is considered to be weak (Mašauskiene, Cesevičiene, 2006). Average degree of softening of ‘Bussard’ wholemeal was 23.8 FU where the degree of softening ‘Zentos’ was 43.0 FU (Table 7). Therefore the dough softening depends on the cultivar genetic characteristics (Mašauskiene, Cesevičiene, 2006) which were confirmed in the present experiment.

**Table 6**

<table>
<thead>
<tr>
<th>Cultivar, Year, factor B</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bussard</td>
<td>2010 17.4</td>
</tr>
<tr>
<td>Zentos</td>
<td>36.8</td>
</tr>
<tr>
<td>Average B</td>
<td>27.1</td>
</tr>
</tbody>
</table>

In 2012, when the protein content in grain was lower (compared with 2010 and 2011), the dough softening of analyzed wholemeal made from both cultivars ‘Bussard’ and ‘Zentos’ was higher—30.1 and 53.3 FU respectively, acquired results agree with report of Varga et al., (2003). The year, cultivar and year–cultivar interaction had a significant (p<0.05) impact on the wholemeal DS12 (Table 3). DS12 mostly depending on cultivars (68.9%), influence of a year was 28.1% while both factors influence was only 2.1%. Protein content is often used as indirect indicator of baking quality—higher protein content means that the dough stability is higher, compare with lower protein content (Kopel, Ingver, 2010).

**Table 7**

<table>
<thead>
<tr>
<th>Cultivar, Year, factor B</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bussard</td>
<td>2010 17.4</td>
</tr>
<tr>
<td>Zentos</td>
<td>36.8</td>
</tr>
<tr>
<td>Average B</td>
<td>27.1</td>
</tr>
</tbody>
</table>

In 2012, when the protein content in grain was lower (compared with 2010 and 2011), the dough softening of analyzed wholemeal made from both cultivars ‘Bussard’ and ‘Zentos’ was higher—30.1 and 53.3 FU respectively, acquired results agree with report of Varga et al., (2003). The year, cultivar and year–cultivar interaction had a significant (p<0.05) impact on the wholemeal DS12 (Table 3). DS12 mostly depending on cultivars (68.9%), influence of a year was 28.1% while both factors influence was only 2.1%. Protein content is often used as indirect indicator of baking quality—higher protein content means that the dough stability is higher, compare with lower protein content (Kopel, Ingver, 2010).

**Figure 1. Correlation coefficient (r) between quality indices**

\[ r^{**} \text{is significant at 99%; } r^{*} \text{at 95% level of probability.} \]

PC—protein content, WA—water absorption; DDT—dough development time; ST—dough stability; DS12—degree of softening.
In our investigation a tendency was found, namely, if the grain protein content is higher the dough formation time is longer, the dough stability is higher, and the dough softening is lower. The wheat wholemeal quality parameters were assed by correlation analysis too (Figure 1). A statistically significant positive correlation was found between PC and ST \( r = 0.972^{**} \), \( R^2 = 0.945 \), a regression equation \( y = 9.192x + 60.48 \), these results are in accordance with studies made by Kopel and Ingver (2010), and Ceseviciene et al. (2012).

Preston (2001) found that the correlation between PC and WA was statistically significant (\( r = 0.51 \)), however in our investigation relationship between PC and WA was higher (\( r = 0.66 \)), but not significant. PC showed high negative relationship between DS12 described by the regression equation \( y = 1.341x + 177.83 \), and correlation coefficient \( r = 0.893^* \), \( R^2 = 0.798 \). Mikos and Podolska (2012) found that higher degree of softening indicated lower dough stability. Our results are in agreement with this as a strong negative relationship was evident between DS12 and ST \( r = -0.878^* \), \( R^2 = 0.771 \), \( y = -0.139x + 12.55 \).

Conclusions

In the present research it was found that wheat cultivars with differences in their genetic as well as weather conditions significantly influence protein formation in grains. Higher protein content was found in the wheat samples in harvest years with warmer growing conditions (2010 and 2011). Winter wheat rheological properties demonstrate that the quality of the studied varieties correspond to the requirements for high-grade wheat for food consumption, and are suitable for wholegrain flour production and bread baking. Winter wheat cultivars ‘Bussard’ and ‘Zentos’ are in different in their farinograph curve shapes. ‘Bussard’ wholemeal had higher water absorption, longer stability time and shorter degree of softening, compared with ‘Zentos’.

Cultivar had a much stronger effect on wholemeal dough water absorption, dough development time and degree of softening than weather conditions in investigations years. The influence of environmental conditions on higher content of protein in winter wheat grain was confirmed as well as wholemeal dough stability time, compared with the cultivar effect. The strong correlation was found between protein content and dough stability time. Degree of softening had negative correlation with protein content. Dough stability correlated negatively with degree of softening.

Acknowledgement

The research has been prepared within the framework of the State Research Programme “Sustainable use of local resources (earth, food, and transport-new products and technologies (NatRes)” project No. 3. „Sustainable use of local agricultural resources for development of high nutritive value food products (Food)”.

References

 SOME CHEMICAL, YIELD AND QUALITY PROPERTIES OF DOMESTIC OAT CULTIVARS

Linda Brunava1,2, Ina Alsina1, Sanita Zute2, Vita Sterna2, Zaiga Vicupe2
1 Institute of Soil and Plant Sciences, Faculty of Agriculture, Latvian University of Agriculture University, Liela street 2, Jelgava, Latvia, e-mail:brunava.linda@inbox.lv
2 State Stende Cereals Breeding Institute, Dizzemes, Dizstende, Talsi district, Latvia

Abstract

Oat (Avena sativa L.) is one of the cereal crops cultivated in climate temperate zones. It is well known as a healthy food in the world, because of its unique biochemical structure. Nowadays the quality of grain for consumers has become important especially in terms of lipids and β-glucan content. The aim of this study was to characterize the yield, volume weight, 1000 kernel weight, husk content and kernel size distribution for two naked and three husked oat cultivars. Some quality analyses were determined, such as protein, starch, lipid and β-glucan contents. Investigations were carried out at the State Stende Cereal Breeding Institute in 2012 and 2013. The obtained results showed signiﬁcant differences among naked and husked oat cultivars in all tested parameters for example lipid content for husked oat cultivars varied from 38.1–66.8 g kg⁻¹, but for naked oat 92.3–108.8 g kg⁻¹; β-glucan content among husked oat cultivars varied from 3.81–3.85 g 100 g⁻¹, but naked oat breeding line ‘33793’ reached 5.07 g 100 g⁻¹. Little variation between years was detected as well. Research showed that naked oat characterized with better quality and insignificant husk content, what is preferable for consumers’, but kernel size uniformity and yielding abilities of husked oat cultivars would be preferable reasons for farmers and food producers to choose them as a raw material. Overall naked oat could be better for food production from consumers’ side, but food producers are made to choose husked oat cultivars because of the requirements of production techniques.

Keywords: naked oat, husked oat, quality.

Introduction

Oat (Avena sativa L.) is an important crop produced in climate temperate zone and distinct among the cereals due to its multifunctional characteristics and nutritional profile. It is used both for human and animal nutrition. Before using in human nutrition, oat was used for medicine purposes. Oat is a nutritious source of protein, carbohydrate, fiber, vitamins and minerals. Currently, discussion on oat grain dietetic value and suitability for the production of functional foods is more frequently mentioned in scientiﬁc literature and scientiﬁc projects (Biel et al., 2009).

Grain yield, volume weight and 1000 kernel weight and husk content are the most important economic traits mentioned by the oat consumers, because the end-product outcome is due to these traits when oat grain is processed (Sadiq Butt et al., 2008). Oat kernel size uniformity is an important parameter for the oat milling industry because the processing of oats for human food generally involves size separation of kernels into different streams before dehulling. Oat spikelets may contain one, two, tree, or more kernels, and the main kernel is always larger than others. Larger oat grains can be dehulled at slower rotor speeds than smaller oat grains, it is because an oat kernel with a larger mass will possess more energy of inertia when impacting the walls of the impact dehuller than smaller oat grains at the same rotor speed. So it is better if oat cultivar is characterized with larger kernel fraction or more of the same size grains (Doehlert et al., 2006b, Doehlert et al., 2004).

Oat breeders through hybridization and selection have improved yielding ability potential of oat varieties; they have developed oat varieties dwarfed in length and more resistant to lodging. On consumers’ side lower standards are set forward regarding biochemical composition of grain: protein, lipids, β-glucan, starch contents in grain, though dietetic value of oats is just due to these traits (Wood, 2007). Protein is considered as the most important nutrient for humans and animals as well. The average protein content of cereal grains covers a relatively narrow range (8–11%) variations, however, are quite noticeable. The main part (approximately 40%) of kernel structural component is starch. Because of its unique properties, starch is important for the textural properties of many foods, in particular bread and other baked goods. It is located only in endosperm and is present in granular form. Oat contains relatively high amounts of lipids (approximately from 7%) compared to other cereal grains (Sadiq Butt et al., 2008).

Among the main compounds associated with health-promoting effects in cereals is dietary fiber which is found only in plant origin foods. It consists of both – soluble and insoluble fiber. Both types are important for human health (Manthey et al., 1999). Water-soluble fiber in cereals is composed of non-starch polysaccharides such as β-glucan also called lichenin. It is presented particularly in barley (3–7%) and oat (3.5–5%), whereas less than 2% β-glucan is found in other cereals. Some of the oat constituents are valuable ingredients or starting materials for several types of products (Brindzova et al., 2008). Oat β-glucan has received the most attention and has a number of potential uses (Wood, 2007).

The aim of this study was to characterize the yield, test weight, 1000 kernel weight, husk content and kernel size distribution for two naked and three husked oat cultivars.

Materials and Methods

Field trials and investigation were carried out at the State Stende Cereals Breeding Institute (SSCBI) in 2012 and 2013. Five local oat cultivars (int. al. two
naked oat and three husked oat cultivars) were used for this research. The soil of the site was sod-podzolic in both, the humus content – 18 g kg\(^{-1}\) (2012), 20 g kg\(^{-1}\) (2013), the soil pH KCl – 6.2 (2012), 6.6 (2013), the available for plants content of phosphorus P – 42 mg kg\(^{-1}\) (2012), 39 mg kg\(^{-1}\) (2013), and that of potassium K – 59 mg kg\(^{-1}\) (2012), 53 mg kg\(^{-1}\) (2013). The pre-crop was barley for both years. All agrotechnical operations were carried out at optimal terms according to the weather conditions during the vegetation period and depending on the plant development phases. Seed rate was 500 seeds per 1 m\(^2\). Before cultivation of the soil, a complex mineral fertilizer was applied: N-51, P-30, and K – 42 kg ha\(^{-1}\). Variants were arranged in four replications with plot size 10 m\(^2\) in a randomized block design. The temperature and moisture conditions provided good oat field germination in 2012 and 2013 and were shown in Figure 1 and Figure 2, respectively.

Harvesting was delayed approximately by ten days because of heavy rainfalls at first decade of August. The opposite situation was in 2013. Precipitation in May was excellent for germination, and lack of moisture in end of vegetation period ripened grains in panicles. Mean samples from all replications (0.5 kg) were taken for testing with Infratec Analyser 1241 (test weight, protein, starch, β-glucan and lipid content) performed at the State Stende Cereals Breeding Institute. 1000 kernel weight was detected by standard method LVS EN ISO 520:2011. Kernel size classifications were carried out with SORTIMAT separator machine. Cleared sample of 100 g to be weighed on a balance accurate to 0.01 g and then placed onto the top sieve. The sieving period was set for 3 minutes, recommended by producers. The sieves were used with diameters of 2.5 and 2.2 mm. With a weighed batch of 100 g, the percentage proportion was then obtained by weighing the individual fractions. Husk content was determined by four samples of 5 g of each cultivar, separating husk from kernel manually and weighed, percentage proportion was calculated. The obtained results were statistically processed by MS Excel program package using the methods of descriptive statistics. ANOVA procedures were used for data analysis.

Results and Discussion

Quality

Nowadays, oat in Latvia has been widely studied as a raw material for human diet. For using oat meals in human nutrition, it is necessary to investigate its quality and thereby suitability. In this study, protein, starch, lipid and β-glucan contents were determined as quality parameters for naked and husked oat cultivars, the results were shown in Table 1.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Protein*</th>
<th>Starch*</th>
<th>Lipids*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naked oat cultivars</td>
<td>S-156</td>
<td>168.8±0.13(^a)</td>
<td>299.8±1.26(^a)</td>
</tr>
<tr>
<td></td>
<td>33793</td>
<td>163.9±0.15(^ab)</td>
<td>349.8±0.87(^b)</td>
</tr>
<tr>
<td>Husked oat cultivars</td>
<td>‘Arta’</td>
<td>114.8±0.10(^b)</td>
<td>462.8±0.17(^ab)</td>
</tr>
<tr>
<td></td>
<td>‘Laima’</td>
<td>100.8±0.06(^b)</td>
<td>458.5±0.20(^ab)</td>
</tr>
<tr>
<td></td>
<td>‘Stendes Dārta’</td>
<td>100.9±0.07(^b)</td>
<td>456.5±0.23(^ab)</td>
</tr>
</tbody>
</table>

\(^a\)difference are significant between naked and husked oat cultivars with the level of p<0.05, \(^b\) trait mean values significantly higher comparing husked and naked oat cultivars, \(^ab\) differences significant between years.

Starch is the major storage carbohydrate of cereals and an important compound for human nutrition. Starch contents of naked oats were significantly (p<0.05)
lower than that of husked oat cultivars. Mean starch content for naked oats was 324.8 g kg⁻¹, but for husked oats – 459.3 g kg⁻¹. Opposite results were reported in the study of Givens et al. (2003), who were found that husked oat cultivars had significantly lower starch contents than that of naked ones (400 g kg⁻¹ and 580 g kg⁻¹, respectively). Starch content in oat grain is subjected to variety, nitrogen treatment and weather conditions. In this study, nitrogen treatment was the same in both years, but chosen varieties and meteorological conditions might affect starch contents according to our results.

Protein and lipid contents of naked oat cultivars were significantly high (p<0.05). Lipids in foods are an important nutritional factor and their profile may play a crucial role as concerns the stability of cereal products (Brindzova et al., 2008). Oat grain has soft kernel and lipid distributed throughout the seed, which makes the milling process more difficult than wheat and corn. To prevent from atmospheric oxidation, the oat is hydrothermally treated before processing (Sadiq Butt et al., 2008). The high lipid content is not desirable for food producers, but crucial component for human diet, because of consistence of vitamin E and fatty acids, which is located in lipids. The highest lipid content (108.8 g kg⁻¹) was observed with naked oat breeding line of S-156. Conciatori et al. (2000) determined mean lipid content for naked oat as 116.4 g kg⁻¹ which was close to our results. The lowest lipid content (66.8 g kg⁻¹) was observed with husked oat variety of Stendes Dārta, similar result obtained with common oat as 72 g kg⁻¹ (Koehler and Wieser, 2013). Lipid content in oat cultivars is strongly dependent on meteorological conditions of sowing year which was indicated by Givens et al. (2003). It was also seen in our research, where lipid content for naked oat cultivars was significantly (p<0.05) higher in 2013. Significant difference (p<0.05) was observed only for naked oat cultivars.

The highest protein content (168.8 g kg⁻¹) among naked oat cultivars was detected with the breeding line of S-156. Among the husked oat cultivars, the highest protein content (114.8 g kg⁻¹) was obtained with the sample of Arta. In the literature, for husked oat, average protein content is reported as 115.0 g kg⁻¹, for naked oat, it was reported as 143.4 g kg⁻¹ (Biel et al., 2009). Protein content in oat grain is dependent on mostly agro-meteorological conditions: variety, nitrogen treatment, sowing date and weather conditions (Givens et al., 2003). That is the reason why significant differences were found between husked and naked oat cultivars, also between varieties and years.

β-glucan, which is a soluble dietary fiber, is an important component of oat grain with health promoting effect. It could have beneficial role in gastrointestinal diseases, lowering of cholesterol level, promoting heart health, preventing diabetes and even cancer (Daou, Zhang, 2012). When naked and husked oat cultivars were compared, husked oat had significantly lower β-glucan content, as shown in

Figure 3. The cultivar of Stendes Dārta had the highest β-glucan content among the husked oat cultivars, like 3.85 g 100 g⁻¹. The naked oat breeding line of 33793 had the highest β-glucan content (5.07 g 100 g⁻¹) among all cultivars studied. In the literature, β-glucan contents for oats were reported between 2.3–8.5 g 100 g⁻¹. Cultivars studied in this research demonstrated average results compared to data reported previously.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Yield*, t ha⁻¹</th>
<th>Husk content*, %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Naked oat cultivars</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-156</td>
<td>3.69±0.33</td>
<td>0.06±0.02</td>
</tr>
<tr>
<td>33793</td>
<td>3.20±0.06</td>
<td>0.10±0.02</td>
</tr>
<tr>
<td><strong>Husked oat cultivars</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arta</td>
<td>4.31±0.31 a</td>
<td>23.23±0.65 ab</td>
</tr>
<tr>
<td>Laima</td>
<td>5.30±0.02 a</td>
<td>26.44±0.18 ab</td>
</tr>
<tr>
<td>Stendes Dārta</td>
<td>5.25±0.04 a</td>
<td>26.14±0.75 ab</td>
</tr>
</tbody>
</table>

*difference are significant between naked and husked oat cultivars with the level of p<0.05, a trait mean values significantly higher comparing husked and naked oat cultivars, b differences significant between years

Husk content of oat grain is about 25–30 % of the seed. The grain is dehulled before use, whereas husk after processing may be used in food industry. Unprocessed husk contains silicate particles, which are harmful to nature and can irritate the mouth, esophagus and gastrointestinal tract (Sadiq Butt et al., 2008). The highest husk content was detected with the cultivar of Laima, as 26.4% (Table 2). Husk contents of naked oat

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Yield*, t ha⁻¹</th>
<th>Husk content*, %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Naked oat cultivars</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-156</td>
<td>3.69±0.33</td>
<td>0.06±0.02</td>
</tr>
<tr>
<td>33793</td>
<td>3.20±0.06</td>
<td>0.10±0.02</td>
</tr>
<tr>
<td><strong>Husked oat cultivars</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arta</td>
<td>4.31±0.31 a</td>
<td>23.23±0.65 ab</td>
</tr>
<tr>
<td>Laima</td>
<td>5.30±0.02 a</td>
<td>26.44±0.18 ab</td>
</tr>
<tr>
<td>Stendes Dārta</td>
<td>5.25±0.04 a</td>
<td>26.14±0.75 ab</td>
</tr>
</tbody>
</table>

*difference are significant between naked and husked oat cultivars with the level of p<0.05, a trait mean values significantly higher comparing husked and naked oat cultivars, b differences significant between years

Husk content of oat grain is about 25–30 % of the seed. The grain is dehulled before use, whereas husk after processing may be used in food industry. Unprocessed husk contains silicate particles, which are harmful to nature and can irritate the mouth, esophagus and gastrointestinal tract (Sadiq Butt et al., 2008). The highest husk content was detected with the cultivar of Laima, as 26.4% (Table 2). Husk contents of naked oat
cultivars were not significant. Yield consists of husk and groat weight. Although naked oat cultivars had low amount of husk, their groat yields were close to the yield of husked oat cultivars. 1000 kernel weight is a parameter which characterizes kernel weight. The results showed that husked oat cultivars have significantly higher (p<0.05) 1000 kernel weight comparing with naked oat cultivars and variation between years was significant as well. Volume weight is most commonly used to evaluate grain quality. Volume weight was significantly (p<0.05) higher for naked oat cultivars also observed by Doehlert et al. (2001), and also its value was influenced by sowing year. 

**Kernel size distribution**

Kernel size uniformity test showed that the distribution of kernel size was dependent mostly on cultivar and two distribution classes (>2.5 and <2.2) on sowing year (Table 3).

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>&gt;2.5</th>
<th>2.5–2.2</th>
<th>&lt;2.2</th>
<th>2012*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naked oat cultivars</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>33793</td>
<td>17.6</td>
<td>42.0</td>
<td>40.4</td>
<td>ab</td>
</tr>
<tr>
<td>S-156</td>
<td>18.5</td>
<td>44.7</td>
<td>36.8</td>
<td>ab</td>
</tr>
<tr>
<td>Husked oat cultivars</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stendes Dārta</td>
<td>47.7</td>
<td>40.5</td>
<td>11.8</td>
<td>ab</td>
</tr>
<tr>
<td>Arta</td>
<td>63.0</td>
<td>27.6</td>
<td>9.4</td>
<td>b</td>
</tr>
<tr>
<td>Laima</td>
<td>36.3</td>
<td>51.9</td>
<td>11.8</td>
<td>b</td>
</tr>
<tr>
<td>Cultivar</td>
<td>&gt;2.5</td>
<td>2.5–2.2</td>
<td>&lt;2.2</td>
<td>2013*</td>
</tr>
<tr>
<td>Naked oat cultivars</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>33793</td>
<td>14.1</td>
<td>39.8</td>
<td>46.0</td>
<td>ab</td>
</tr>
<tr>
<td>S-156</td>
<td>12.1</td>
<td>42.8</td>
<td>45.1</td>
<td>ab</td>
</tr>
<tr>
<td>Husked oat cultivars</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stendes Dārta</td>
<td>55.7</td>
<td>40.3</td>
<td>4.0</td>
<td>b</td>
</tr>
<tr>
<td>Arta</td>
<td>67.4</td>
<td>27.1</td>
<td>5.4</td>
<td>b</td>
</tr>
<tr>
<td>Laima</td>
<td>44.5</td>
<td>49.8</td>
<td>5.7</td>
<td>b</td>
</tr>
</tbody>
</table>

*difference are significant between husked and naked oat cultivars with the level of p<0.05, a trait mean values significantly higher comparing husked and naked oat cultivars, b differences significant between years.

With larger kernels (>2.5 mm) characterized as husked oat cultivars. For example 63.0% of kernels in 2012 and 67.4% of kernels in 2013 were observed in class of >2.5 mm for the cultivar of Arta. For food production, such cultivar with larger grains is more suitable than others, because it requires lower rotor speed in dehulling process (Doehlert et al., 2004). As the husked oat spikelets may contain 1 to mostly 3 florets, naked oat spikelets consists of up to 12 florets (Doehlert et al. 2006a). It means that the kernels of naked oat cultivars are smaller comparing with husked oat. As we mentioned that kernel size distribution is quite dependent on cultivar, the results of other scientists were much different from ours. Doehlert et al. (2004) worked with four sieve classes, like 3.18, 2.58, 2.38, 1.98 mm slot sieves. The most of husked cultivars were characterized as small grains (2.38–1.98 mm slot) in that research. Kernel size uniformity is an important character for food producers, but it is hard to find cultivars with required quality parameters, such as large grains.

**Conclusions**

For all tested parameters, there were significant (p<0.05) differences between naked and husked oat cultivars. The naked oat cultivars exhibited better quality parameters, for example they had the highest protein and the lowest starch contents. The lipid contents of naked oat cultivars was higher than the reported literature findings, however β-glucan level failed to meet expectations and average value was obtained in studied years. Productivity of tested cultivars showed that yield, husk content and 1000 kernel weight results of husked oat cultivars were higher than naked oat cultivars. Besides, volume weight results of naked oat cultivars were higher than that of husked oat cultivars. Such parameters, like larger grain size, yield and lower lipid content could be main reasons for food producers to choose husked oat cultivars. When the low processing requirements is considered, the naked oat cultivars can be more valuable for food industry.

**Acknowledgment**

This work was supported by the Latvian State Research Program in Agro – biotechnology, and the European (ESF – Social Fund) co-financed the Project Nr. 1DP/1.1.1.2/13/APIA/VIAA/032.

**References**

LIPID COMPOSITION OF OAT GRAIN GROWN IN LATVIA

Vita Stern1, Sanita Zute2, Linda Brunava1, Zaiga Vicupe2

1 Department of Agro-ecological Research, State Stende Cereals Breeding Institute, „Dizzones“, Dizstende, Libagia parish, Talsi County, Latvia, e-mail: vitasterna@inbox.lv
2 Department of Cereal Breeding, State Stende Cereals Breeding Institute, Dizzones, Dizstende, Libagia parish, Talsi County, Latvia

Abstract

Oat (Avena sativa L.) is a well known annual crop in temperate climates. It is recognised as a healthy food containing significant amounts of fat-soluble vitamin E and polyunsaturated fatty acids in the world. There are few investigations of lipid composition in connection with human health in Latvia. Therefore the aim of this study was to characterize the composition of lipids of same oat varieties to grown in Latvian condition. Investigations were performed at the State Stende Cereal Breeding Institute. In the studied samples content of fat, composition of fatty acids and vitamin E (α-tocopherol) were determined, same as ratio most significant for human health like PUFA/SFA and ω-3/ω-6 were calculate. The total fat was made by Soxlet method, fatty acids by LVS CEN ISO/TS 17764-1:2007 and content α-tocopherol determination was made using high-performance liquid-chromatography. The high concentration was determined as mg kg dry matter. The obtained results showed a wide range of fat content among varieties, it varied from 4.9 to 10.5 g 100 g−1. The content of α-tocopherol in oat grain was determined 8.5–12.3 mg kg−1, and the sum of unsaturated fatty acids accounted 78–81.5% of total fatty acids content. The ratio P/S varied from 2.2 to 2.4. Result of evaluation leads to conclusion that lipid of oat grain are rich with biologically significant substances.

Keywords: oat for human health, fat content, fatty acids, vitamin E.

Introduction

Oat is recognised as a healthy food containing significant amounts of fat-soluble vitamin E and polyunsaturated fatty acids in the world. The fat content of oat grain is the highest among grains and varied from 4.2 to 11.8 g 100 g−1, in comparison with wheat (2.1–3.8 g 100 g−1), rice (2.0–3.1 g 100 g−1), millet (4.0–5.5 g 100 g−1), barley (3.3–4.6 g 100 g−1), rye (2.0–3.5 g 100 g−1) (Zhou, 1999). Total lipid in oat grains averages 4–6% when based on relatively few varieties. However, larger ranges are apparent when more genotypes are considered, the range of 3.1 to 11.6% was found among more than 4 000 US entries in the world collection (Zhou, 1999). A high lipid content of 15.5% in a US experimental line of groats was reported by Hartunian – Sowa and White, 1992. Morphologically, oat can be classified as husked and naked; naked oat is nutritionally richer than the common husked oat.

Vitamin E activity is provided by the tocopherols and tocotrienols, which together create tocals. Tocals are fat soluble and form part of the total lipid. The vitamin activity of tocals is associated with their antioxidant function in vivo. The highest level of vitamin E activity is produced by α-tocopherol, following by β-tocopherol and γ-tocotrienol, which have 40% and 30% of the activity of α-tocopherol, respectively on an equal weight basis (Welch, 1995; Zielinski et al., 2001; Jackson et al., 2008). Alpha-tocopherol is a major antioxidant component in crude oat unaltered when the lipid is refined (Webster, 1986). Among the Latvian genotypes, the variety ‘Arta’ had the highest α-tocopherol concentration – 7.5 mg kg−1, and there was no significant difference between years (Berga et al., 2012).

The fatty acid composition of oat lipids is bound as to oat breeders so to nutritionists because of the nutritional significance of fatty acids. Separate fatty acids (FA) also have different impact on human health. Many studies estimate the beneficial effects of polyunsaturated (PUFA) and monounsaturated fatty acids (MUFA) (Chillard et al., 2000). Linoleic, oleic and palmitic acids are major oat lipid constituents (Brindzova et al., 2008). The proportion of unsaturated fatty acids is about 75% of total and content of palmitic acid as main of saturated fatty acids determined 14–17% (Saastamoinen, 1989).

To compare the potential impact of fat on human health often used fatty acids ratio in the scientific literature. The most commonly used is ratio – sum of polyunsaturated fatty acids to sum of saturated fatty acids – PUFA/SFA. World Health Organization (2003) recommended ratio PUFA/SFA must be higher than 0.4. The decrease in ratio between n-6 and n-3 FA, as well as increase in ratio between stearic / palmitic acids (C18:0 / C16:0) and oleic / stearic acids (C18:1c / C18:0) are desirable for the prevention of number of diseases (Gebauer et al., 2005). The lipid content of oat grain depends on genetic and environmental factors as well as the method of determination (Zhou, 1999). The limiting heritabilities for fat content is high and polygenic (Karow, Rosberg, 1984).

The aim of this study was to characterize the composition of lipids of same oat varieties to grown in Latvian condition.

Materials and Methods

Investigations were performed at the State Stende Cereal Breeding Institute. The material consisted of 7 oat genotypes (int. al. 4 husked and 3 naked). In the studied samples fat, α-tocopherol content and fatty acids composition were determined.

Fat content was made by Soxlet method.

Vitamin E. Mean samples from all replications (0.5 kg) were taken and milled for α-tocopherol determination in the Laboratory of Food and Environmental Investigations of the Institute of Food Safety, Animal
FOODBALT 2014

Health and Environment BIOR, using high-performance liquid-chromatography. The method is often used for detection of α-tocopherol in diet samples. Absorption was measured at 292 nm. Chromatography was carried out in a C18 column using methanol / water liquid (98 / 2 v/v). The concentration was determined as mg kg⁻¹ in dry matter.

Fatty acids. Previously homogenized samples were prepared for GLC (gas-liquid chromatography) analysis using direct saponification with KOH/methanol followed by a derivatization with (trimethylsilyl) diazomethane. Alltech AT-FAME analytical column was used. The carrier gas (He) flow rate was 2 mL min⁻¹. The injector and detector temperatures were 225 °C and 250 °C, respectively. Peaks were identified using standard mixture Supelco FAME Mix C4-C24, Sigma Aldrich. The concentration was determined as % of total fatty acids.

The content of different fatty acids (FA) was calculated according to formulas 1–3:

\[ \text{SFA} = \sum \text{C14:0; C16:0; C18:0; C20:0; C22:0} \]  
\[ \text{MUFA} = \sum \text{C16:1; C18:1; C20:1} \]  
\[ \text{PUFA} = \sum \text{C14:0; C16:0; C18:0; C20:0; C22:0} \]

The obtained results were statistically processed using descriptive statistics in the MS Excel software package. Correlation defined as medium close if 0.5 < r < 0.79. Differences between the groups were tested for significance (p<0.05) by ANOVA.

Results and Discussion

Obtained results of fat and α-tocopherol contents in analyzed oat grain samples assumed in the Table 1.

Table 1

Average fat and α-tocopherol contents of oat grain

<table>
<thead>
<tr>
<th>Genotypes /Lines</th>
<th>fat, g 100 g⁻¹</th>
<th>α-tocopherol mg kg⁻¹</th>
<th>% of fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naked oat cultivars</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-156</td>
<td>8.9</td>
<td>10.3</td>
<td>0.012</td>
</tr>
<tr>
<td>33793</td>
<td>7.9</td>
<td>10.2</td>
<td>0.013</td>
</tr>
<tr>
<td>33805</td>
<td>10.7</td>
<td>9.0</td>
<td>0.009</td>
</tr>
<tr>
<td>Husked oat cultivars</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘St. Līva’</td>
<td>4.0</td>
<td>9.0</td>
<td>0.019</td>
</tr>
<tr>
<td>‘Laima’</td>
<td>5.65</td>
<td>4.2</td>
<td>0.007</td>
</tr>
<tr>
<td>‘Arta’</td>
<td>5.32</td>
<td>5.8</td>
<td>0.011</td>
</tr>
<tr>
<td>‘St. Dārta’</td>
<td>5.67</td>
<td>3.3</td>
<td>0.012</td>
</tr>
</tbody>
</table>

As showed in the table 1, fat content same as content of α-tocopherol in grain samples differed significantly among oat varieties (p<0.05). Varieties of husked oat ‘Stendes Līva’ and ‘Arta’ had smallest fat content – 4.0 and 5.32 g 100 g⁻¹ respectively. The richest in fats was grains of naked oat breeding line ‘338055’. Data of other investigations show that fat content of oat varied from 5–9% (Morrison, 1979) and 4.2–11.8 g 100 g⁻¹ (Zhou, 1999).

Content of α-tocopherol in oats determined from 3 to 12.3 mg kg⁻¹, the richest was grains of breeding lines ‘S-156’ and ‘33793’ with average α-tocopherol contents 10.3 mg kg⁻¹ and 10.2 mg kg⁻¹ respectively. Relatively richest with vitamin E was fat of variety ‘St.Līva’, with α-tocopherol concentration 0.019% of fat content. Previous investigations among the Latvian genotypes show that the variety ‘Arta’ had the highest α-tocopherol concentration – 7.5 mg kg⁻¹ (Berga, 2012). Oat genotypes bred in Latvia did not show a marked difference when compared with material of foreign origin. The difference between husked and naked oat genotypes can be explained by the differences in sample structure.

Correlation between fat and α-tocopherol content reflected in Figure 1.

Figure 1. Correlation between fat and α-tocopherol content in oat grain samples

Correlation between fat and α-tocopherol content is not strong r=0.63, in husked oats correlation was calculate r=0.83, but in naked oats r=0.57. Our study confirms that just 39% of α-tocopherol changes could explain with fat content in oat grains, more than 60% explain with other factors such as oats variety impact. The lipid fraction of the oat grain determines in large measure nutritional quality via the fatty acid composition. The content and degree of unsaturation of the oat total fatty acid was higher in winter sown than in spring sown crops, indicating that low temperatures cause a higher synthesis of unsaturated fatty acids in oats (107).

Fatty acid composition of husked oat grain same as ratio PUFA/SFA, C18/C16; C18:1/C18 assumed in Table 2 and fatty acid composition of naked oat grain with ratios assumed in Table 3.

Obtained results showed that content of oleic acid was determined 36.2−40.4% and content of linoleic acid 38.4−41.6%. Data of other investigations show that content of oleic acid in oat grain varieties determined from 37.2 to 42.1% and content of linoleic acid 38.6–42.5% (Saastamoinen, 1989).
The main saturated fatty acids increased cholesterol level in human blood are myristic acid and palmitic acid. Results of investigation showed that myristic acid determined 0.2% in oats of ‘Laima’ and content of palmitic acid in oat varieties determined 15.5–17.4%. The reviewed data showed higher content of myristic acid (0.2–4.9%) and palmitic acid (13.2–28.0%) in oat grains.

The evaluation of ratios significant for human health leads to conclusion that lipid of oat grain are rich with biologically significant substances.

As mentioned before, the increase in ratio between stearic / palmitic acids (C18:0 / C16:0) and oleic / stearc acids (C18:1c / C18:0) are desirable for the prevention of number of diseases. The calculated ratio C18:0 / C16:0 varied from 0.10 to 0.15 and higher was calculated in husked oat breeding line ‘33793’. Evaluation of ratio C18:1 / C18:0 showed that more healthy are husked oat samples ‘Laima’ same as naked oat samples ‘S-156’.

Assumed saturated, mono unsaturated and polyunsaturated fatty acids in oat grains are showed in the Figure 2.

![Figure 2. Proportions of fatty acids groups in oat grains](image)

The sum of saturated fatty acids varied from 18.2% to 19.6% of total fatty acids and it was not differed among varieties (p>0.05). As showed in Figure 2, the sum of monounsaturated fatty acids accounted 40.4–44.4% and the sum of polyunsaturated fatty acid 37–41.1% of total fatty acids.

The sum of unsaturated fatty acids accounted 78–81.5% of total fatty acids. Saastamoinen et al. (1989) had determined mean content of unsaturated fatty acids about 75%, content of palmitic acid 14–17% for oat varieties which was close to ours.

**Conclusions**

The fat content was determined from 4 g 100 g⁻¹ to 10.7 g 100 g⁻¹ in naked oat grain samples. There were observed significant (p<0.01) difference in fat content among oat varieties.

Content of α-tocopherol in oats determined from 3 to 12.3 mg kg⁻¹, the richest was grains of naked oats breeding lines ‘S-156’ and ‘33793’ with average α-tocopherol contents 10.3 mg kg⁻¹ and 10.2 mg kg⁻¹ respectively.

The sum of unsaturated fatty acids accounted 78.0–81.5% of total fatty acids. Result of evaluation leads to conclusion that lipid of oat grain are rich with biologically significant substances.

**Acknowledgment**

This work was supported by the Latvian State Research Program in Agro – biotechnology, and the European
(ESF – Social Fund) co-financed the Project Nr. IDP/1.1.1.2/13/APIA/VIAA/032.

References
OAT HULLS AND SEA BUCKTHORN POMACE – A POTENTIAL SOURCE OF ANTIOXIDANTS FOR HEMPSSEED OIL

Sindija Zeberga, Maija Strele, Anna Vojevodova, Inese Mierina, Mara Jure

Institute of Technology of Organic Chemistry, Faculty of Material Science and Applied Chemistry, Riga Technical University, Str. Paula Valdiena 3, Riga, Latvia, e-mail: mara@kff.rtu.lv

Abstract

Hempseed oil, as well as other oils containing polyunsaturated fatty acids, is subjected to oxidation processes caused by air, heat or light. Usually these processes are suppressed by addition of various synthetic phenol type antioxidants. The aim of current research was to increase oxidative stability of vegetable oils with natural antioxidants. Herein, we demonstrate that the oxidative stability can be increased with natural antioxidants present in oat hulls and sea buckthorn pomace – by-products of food processing. Extracts from pomace and hulls were prepared by maceration of ground plant material in the hempseed oil. The extraction was accelerated by ultrasound. It was established that the highest amount of polyphenols in extracts of both plant materials can be achieved within 30 min; further increase of extraction time sometimes even reduced the total amount of polyphenols. The highest amount of polyphenols in sea buckthorn extracts was 7.73±0.29 mg GAE 100 g⁻¹, but in oat hull extracts – 4.63±0.21 mg GAE 100 g⁻¹, when extracts were prepared by ultrasonification of hempseed oil containing 5% wt of plant material. Various amounts of plant material additives were used for extraction under optimized conditions. The highest antioxidant activity (expressed as ratio of time when peroxide value of sample and blank reaches 48 meq O₂ kg⁻¹) – 1.51±0.05 and 1.40±0.06 to 1.44±0.02, respectively – had extracts obtained from 1% wt additive of sea buckthorn pomace or 2.5–5.0% wt additive of oat hulls. The prepared hempseed oil extracts demonstrated higher oxidative stability than hempseed oil containing 0.02% additive of synthetic antioxidant BHT.

Keywords: hempseed oil, oat hull, sea buckthorn pomace, oxidative stability.

Introduction

Oats (Avena sativa L.) demonstrate antithromogenic properties in in vitro assays, animal experiments and human studies, exhibit anti-inflammatory and antioxidative action (Andersson, Hellstrand, 2012; Pazyar et al., 2012). Oats reduce coronary heart disease (Harris, Kris-Etherthon, 2010; Truswell, 2002) via different mechanisms – decreasing serum low-density lipoprotein cholesterol, blood pressure and improving glucose and insulin response (Harris, Kris-Etherthon, 2010). Due to the skin protecting effect against ultraviolet rays, oats are used in cosmetic formulations (Pazyar et al., 2012; Kurtz, Wallo, 2007). Unique oat polyphenols – avenanthramides – have strong antioxidant activity in vivo and in vitro, exhibit anti-inflammatory, antiproliferative and anti-itching activity, which may provide additional protection against coronary heart disease, colon cancer and skin irritation (Meydani, 2009; Guo et al., 2009).

Sea buckthorn (Hippophae rhamnoides L.) is widely used due to its biological activity: oil (Kumar et al., 2011), fruits and leaves (Suryakumar, Gupta, 2011; Xu et al., 2011) are used to treat cancer and disorders of skin, as well as cerebral-cardiovascular and immune systems. Sea buckthorn is applied to lower cholesterol, platelet aggregation, blood pressure and blood sugar (Xu et al., 2011). The oil absorbs ultraviolet light and promotes healthy skin (Stobdan et al., 2013; Vernet, 2006).

Due to the great biological properties of oats we studied oat grains as a source of natural antioxidants to enhance oxidative stability of various vegetable oils. Previously we have clarified, that additives of oat grains improve the oxidative stability of vegetable oils more than 3.5 times (Jure et al., 2010); other authors (Xing, White, 1997) have established that methanolic extracts of oat hulls dramatically reduced the formation of peroxides during storage of the soybean oil. Our current studies are devoted to the examination of oat hulls as an alternative source of antioxidants for stabilization of hempseed oil; the impact of natural antioxidants extracted from oat hulls and pomace of sea buckthorn is compared. The usage of these sources of natural antioxidants is sustainable and economically beneficial, as both of them are by-products of food industry.

Materials and Methods

Materials

Hempseed oil (HO) was obtained by cold-pressing method at DUO AG Ltd. Sea buckthorn pomace (SB) was supplied by farm Puntingi as the by-product of juice manufacturing. Oat hulls (OH) were purchased from Rigas Dzirnavnieks JSC.

Preparation of hempseed oil extracts

Ground sea buckthorn pomace or oat hulls were mixed with hempseed oil [ratio of plant material and hempseed oil was 1:99, 1:39 or 1:19 (wt:wt)]. The mixture was treated with ultrasound (for the time of ultrasonification see Table 1). The temperature was 16 to 29 °C in the sonification bath. The plant material after the extraction was separated by centrifugation at 3000 rpm. Each extract was prepared triplicate.

Preparation of the extracts of polyphenols

The hempseed oil extract (4 g) was shaken with 80% ethanol (10 mL) at room temperature in dark for 24 h by orbital shaker Biosan OS-10. The ethanol extract was used for further analysis of polyphenols according to the Folin-Ciocalteu method (Mierina et al., 2011). The total amount of phenolic compounds (TAP) was calculated from calibration curve (R²=0.9936) and was expressed as mg of gallic acid equivalents per 100 g of oil (mg GAE 100 g⁻¹ oil); calibration curve consisted
from 5 points and was linear in the range of obtained results. The absorption of all solutions was measured with single beam scanning UV/Visible spectrometer 

**Camspec M501.**

**Antioxidant activity**

The hempseed oil extracts of oat hulls and sea buckthorn pomace were kept under accelerated oxidation conditions in thermostat at 40 °C in dark. The samples (30 g) were filled in Petri dishes with diameter 10 cm. The oxidation process was characterized with peroxide value. Antioxidant activity (AA) was expressed according to the equation:

\[
AA = \frac{t_{\text{extract}}}{t_{\text{blank}}} 
\]

where

- \( t_{\text{extract}} \) is the time when peroxide value reaches 48 meq O\(_2\) kg\(^{-1}\) for hempseed oil extract of plant material and hempseed oil without additive, respectively.

Analogously the impact of 0.02% butylated hydroxytoluene (BHT) additive on oxidative stability of hempseed oil was detected.

**Antiradical activity**

2,2-Diphenyl-1-picrylhydrazyl DPPH (2 mL, 200 μM solution in isooctane) and hempseed oil extract of sea buckthorn or oat hulls (2 mL, 40, 30, 20 or 10 mg mL\(^{-1}\) solution in isooctane) were mixed; after 30 min absorption (\(A_{\text{abs,blank}}\)) of solution was measured at 515 nm. The blank (\(A_{\text{abs,blank}}\)) was prepared as above mentioned, pure isooctane was used instead of the solution of hempseed oil extract. The inhibition of DPPH (INH, %) was expressed according to the equation:

\[
INH = \frac{A_{\text{abs,blank}} - A_{\text{abs,extract}}}{A_{\text{abs,blank}}} \times 100\% 
\]

In order to find out the concentration that inhibits 50% of DPPH, the curves were constructed between the concentration of hempseed oil extract in isooctane and inhibition of DPPH (%).

**Peroxide and acid values**

Peroxide and acid values were determined according to standard LVS EN ISO 3960 and LVS EN ISO 660, respectively.

**Statistical analysis**

Statistical analysis was carried out using Microsoft Excel software package. Standard deviation was calculated by linear least squares regression. All measurements were carried out at least triplicate.

**Results and Discussion**

Hempseed oil is characterized with high amount of polyunsaturated (especially, linoleic) fatty acids. The ratio of \(n\)-6 and \(n\)-3 fatty acids is 3:1; therefore implementation of hempseed oil into the diet would shift the ratio to the recommendations of various nutrition societies (Matthäus, Brühl, 2008). The oil is widely used in cosmetic formulations due to the presence of \(\gamma\)-linolenic acid (Da Porto et al., 2012). Besides that, the oil contains high amount of tocopherol – even higher than in flax and canola seed oil (Teh, Birch, 2013). Unfortunately it has to be taken into consideration that the linoleic and linolenic acids are highly susceptible to oxidation. Also the presence of high amounts of chlorophyll promotes the oxidation (Matthäus, Brühl, 2008).

Both sea buckthorn and oat are rich sources of antioxidants. Sea buckthorn oil (Kumar et al., 2011), fruits and leaves (Suryakumar, Gupta, 2011) are well known sources of tocopherols, tocotrienols, carotenoids, phytosterols, vitamin C, phenolic compounds and trace elements. Main oat antioxidants are vitamins, carotenoids, phenolic compounds including phenolic acids and flavonoids, phytoestrogens, phytosterols, inositol phosphates, glutathione and melatonin (Zielinski et al., 2012; Peterson, 2001). Oat is almost exclusive source of \(N\)-cinnamoylanthranilates called avenanthramides (Peterson, 2001). Oat hulls and sea buckthorn pomace can increase oxidative stability of polyunsaturated hempseed oil, as well as make it even more advantageous for cosmetic purposes.

Usually extracts of oat and sea buckthorn antioxidants are prepared using various organic solvents. Such extraction procedure has several disadvantages – flammability of organic solvents and necessity to evaporate solvents require special equipment, but solubility of such extracts in vegetable oils may be problematic. Besides that toxicity and harmfulness of organic solvents must be kept in mind when the extract is envisaged for food or cosmetic industry. We already described preparation of “ready to use” extracts having increased oxidative stability obtained from sea buckthorn seeds and pomace (Seržane et al., 2012) and grains of oats (Jure et al., 2010) by extraction with vegetable oils. Usually the extraction was carried out at 40 °C in dark for 24 h or the extract was prepared using various cold-pressing techniques.

It is well known that antiradical activity of hempseed oil can be increased if the seeds before the oil extraction are treated with ultrasound for 20 to 40 min (Da Porto et al., 2013). In order to enhance the extraction as well as to use conditions that can increase the oxidative stability of hempseed oil we examined ultrasound accelerated process. Ground plant material and hempseed oil were mixed in ratio 1:19 (in the previous studies we clarified that the optimal ratio for various plant materials, including oat grains and sea buckthorn pomace was 5% additive to the vegetable oil) and treated with ultrasound for various time from 10 min to 1 h (see Table 1). Ultrasonification had small impact on the quality of hempseed oil – immediately after the extraction peroxide and acid values of extracts were similar in most of the cases; ultrasonification slightly increased peroxide value of extracts in comparison with samples prepared by cold-pressing (HO-2, SB-11). Both the amount of plant material
additive and the duration of ultrasonification had negligible effect on the peroxide value. The effectiveness of extraction was characterized by the total amount of polyphenols (see Table 2) – ultrasonification raised TAP value from 4.8 to 7.7 mg GAE 100 g⁻¹ oil in case of sea buckthorn extracts (within 30 min) and to 5.5 mg GAE 100 g⁻¹ oil for extracts of oat hulls (within 20 min).

When irradiation was carried out just for 10 min (OH-1, SB-1), TAP did not change in comparison with sonicated hempseed oil without any additive (HO-1), most probably due to the partial degradation of antioxidants both in the plant material and hempseed oil. The continued (more than 30 min) ultrasonification of the samples generally slightly decreased the total amount of polyphenols: e.g., the difference between cold-pressed hempseed oil and sonicated (for 30 min) oil was 0.6 mg GAE 100 g⁻¹ of oil. The increase of TAP during the short ultrasonification can be explained both with more complete extraction process and formation of endogenous antioxidants, the nature and amount of which may depend on sonication time (Da Porto et al., 2013). TAP increased with the amount of plant material used for the extraction; the difference of the TAP was about 2.4 mg GAE 100 g⁻¹ of oil for the extracts obtained from 1 and 5% additive of sea buckthorn (SB-7 and SB-3). The amount of oat hulls additive did not show strong regularity and had less impact on TAP – the maximal value was reached with 5% additive by 60 min ultrasonification, but similar TAP had extract obtained only within 20 min.

Table 1

| Hempseed oil extracts of sea buckthorn pomace and oat hulls |
|---------------------------|----------------|----------------|
| Abbr.  | AmA | TU | PV | AV |
| OH-1  | 5.0 | 10 | 5.24±0.36 | 5.05±0.32 |
| OH-2  | 5.0 | 20 | 6.06±0.20 | 4.51±0.09 |
| OH-3  | 5.0 | 30 | 5.59±0.59 | 4.44±0.05 |
| OH-4  | 5.0 | 40 | 6.47±0.61 | 4.25±0.04 |
| OH-5  | 5.0 | 50 | 3.88±1.31 | 4.48±0.14 |
| OH-6  | 5.0 | 60 | 4.37±0.96 | 4.35±0.07 |
| OH-7  | 1.0 | 30 | 5.20±0.63 | 4.26±0.01 |
| OH-8  | 2.5 | 30 | 3.07±0.13 | 4.48±0.12 |
| SB-1  | 5.0 | 10 | 4.59±0.42 | 4.64±0.07 |
| SB-2  | 5.0 | 20 | 4.05±0.27 | 4.43±0.17 |
| SB-3  | 5.0 | 30 | 4.21±0.41 | 4.62±0.41 |
| SB-4  | 5.0 | 40 | 4.48±0.88 | 4.46±0.01 |
| SB-5  | 5.0 | 50 | 4.42±0.62 | 4.28±0.04 |
| SB-6  | 5.0 | 60 | 4.09±0.27 | 4.43±0.05 |
| SB-7  | 1.0 | 30 | 5.82±0.73 | 4.39±0.16 |
| SB-8  | 2.5 | 30 | 4.52±0.32 | 4.45±0.04 |
| HO-1  | 0   | 30 | 5.74±0.94 | 4.40±0.40 |
| HO-2  | 0   | 0  | 4.32±1.54 | 4.79±0.88 |
| SB-11 | 5.0 | 0  | 2.38±0.53 | 3.45±0.85 |

AmA – Amount of additive in the mixture of plant material and hempseed oil, %; TU – Time of the ultrasonification, min. * – The extract was prepared according to the known cold-pressing method (Poiss, 2004).

In order to evaluate the impact of extraction conditions and the amount of oat hulls and sea buckthorn pomace on the oxidative stability of hempseed oil, all samples were kept under accelerated oxidation conditions in Petri dishes at 40 °C. The accelerated oxidation was continued until the peroxide value of the sample reached at least 48 meq O₂ kg⁻¹. The curves of peroxide values were used to estimate antioxidant activity of the prepared extracts – in most of the cases antioxidant activity varied from 1.3 to 1.5 (see Table 2); such activity is considered as low or very low (Ramamoorthy, Bono, 2007). Nevertheless the effect of sea buckthorn and oat hulls is comparable to the activity of synthetic antioxidant BHT – 0.02% additive of BHT increases oxidative stability of hempseed oil 1.5 times. The oxidative stability of hempseed oil extracts did not increase proportionally to TAP value – extracts of sea buckthorn pomace demonstrated strong antioxidant-prooxidant effect; any correlation between TAP and AA was not observed for the extracts of oat hulls. The acid values of all samples at the end of experiments did not exceed 5 mg KOH g⁻¹. The extracts that were prepared under ultrasound accelerated conditions demonstrated better AA and higher TAP than the extract SB-11 obtained by cold-pressing (method described by Poiss (2004)) of mixture of hempseeds and sea buckthorn pomace.

Table 2

| TAP and antioxidant activity of extracts of sea buckthorn pomace and oat hulls |
|-------------------------------|----------------|----------------|
| Abbreviation                  | TAP            | AA             |
| OH-1                          | 4.85±0.37      | 1.50±0.03      |
| OH-2                          | 5.47±0.10      | 1.30±0.02      |
| OH-3                          | 4.63±0.21      | 1.44±0.02      |
| OH-4                          | 4.65±0.04      | 1.42±0.17      |
| OH-5                          | 4.74±0.09      | 1.37±0.04      |
| OH-6                          | 5.58±0.28      | 1.36±0.04      |
| OH-7                          | 5.32±0.32      | 1.32±0.02      |
| OH-8                          | 4.60±0.24      | 1.40±0.06      |
| SB-1                          | 4.86±0.17      | 1.26±0.03      |
| SB-2                          | 7.07±0.19      | 1.27±0.06      |
| SB-3                          | 7.73±0.29      | 1.32±0.02      |
| SB-4                          | 6.68±0.20      | 1.31±0.04      |
| SB-5                          | 6.63±0.26      | 1.37±0.04      |
| SB-6                          | 6.73±0.15      | 1.35±0.04      |
| SB-7                          | 5.33±0.24      | 1.51±0.05      |
| SB-8                          | 6.08±0.88      | 1.42±0.02      |
| HO-1                          | 4.86±0.15      | –              |
| HO-2                          | 5.51±0.05      | 1.00           |
| SB-11                         | 4.78±0.44      | 0.75±0.02      |

In order to evaluate the impact of extraction conditions and the amount of oat hulls and sea buckthorn pomace on the oxidative stability of hempseed oil, all samples were kept under accelerated oxidation conditions in Petri dishes at 40 °C. The accelerated oxidation was continued until the peroxide value of the sample reached at least 48 meq O₂ kg⁻¹. The curves of peroxide values were used to estimate antioxidant activity of the prepared extracts – in most of the cases antioxidant activity varied from 1.3 to 1.5 (see Table 2); such activity is considered as low or very low (Ramamoorthy, Bono, 2007). Nevertheless the effect of sea buckthorn and oat hulls is comparable to the activity of synthetic antioxidant BHT – 0.02% additive of BHT increases oxidative stability of hempseed oil 1.5 times. The oxidative stability of hempseed oil extracts did not increase proportionally to TAP value – extracts of sea buckthorn pomace demonstrated strong antioxidant-prooxidant effect; any correlation between TAP and AA was not observed for the extracts of oat hulls. The acid values of all samples at the end of experiments did not exceed 5 mg KOH g⁻¹. The extracts that were prepared under ultrasound accelerated conditions demonstrated better AA and higher TAP than the extract SB-11 obtained by cold-pressing (method described by Poiss (2004)) of mixture of hempseeds and sea buckthorn pomace.
Antiradical activity against DPPH was measured for the extracts obtained by 30 min ultrasonification (Table 3). The most common solvent for DPPH analysis is alcohol (mainly ethanol or methanol). Nevertheless extensive studies of the mechanism of antioxidant interaction with free radicals have revealed that this mechanism strongly depends on the properties of the solvent. It is established that the reaction of phenols with DPPH in alcoholic media goes as sequential proton loss and electron transfer (SPLLET) (Litwinienko, Ingold, 2003; Foti et al., 2004). It is well known that oxidation of vegetable oils proceeds via homolytic cleavage of C-H bond mainly at bis-allylic and allylic positions of fatty acid moiety (Belitz et al., 2004). In order to avoid SPLLET process during DPPH analysis we carried out our experiments in isooctane – a solvent that supports hydrogen atom transfer (HAT) mechanism (Litwinienko, Ingold, 2007). Hempseed oil extracts of oat hulls demonstrated better antiradical activity against DPPH in comparison to extracts of sea buckthorn pomace (Table 3).

Moderate correlation existed between inhibition of DPPH and total amount of polyphenols in extracts of sea buckthorn pomace – the antiradical properties of these extracts generally rose with the increase of TAP. Such correlation was not observed in case of extracts of oat hulls. Medium linear correlation was found both for hempseed oil extracts of sea buckthorn pomace and oat hulls between inhibition of isolectronic peroxyl and antioxidant activity of extracts; the first one was expressed as antioxidant activity and the second – as concentration (mg mL\(^{-1}\)) of oil extract in isooctane that inhibits 50% of DPPH with initial concentration 100 μM (abbreviated as IC\(_{50}\)).

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Antiradical activity of hempseed oil extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hempseed oil extracts of oat hulls</td>
</tr>
<tr>
<td></td>
<td>Hempseed oil extracts of sea buckthorn pomace</td>
</tr>
<tr>
<td>Extract</td>
<td>IC(_{50}), mg mL(^{-1})</td>
</tr>
<tr>
<td></td>
<td>Extract</td>
</tr>
<tr>
<td>OH-3</td>
<td>8.4±0.2</td>
</tr>
<tr>
<td>OH-7</td>
<td>8.0±0.1</td>
</tr>
<tr>
<td>OH-8</td>
<td>7.7±0.0</td>
</tr>
</tbody>
</table>

Conclusions
Our studies demonstrate that ultrasound is an effective mean to accelerate extraction of sea buckthorn pomace and oat hulls with hempseed oil. Even within 30 min the maximal total amount of polyphenols can be reached and such extracts demonstrate acceptable oxidative stability. Luck of direct correlation between total amount of polyphenols and antioxidative activity, as well as between TAP and antiradical activity of extracts let us suggest that beside polyphenols other compounds present in extracts have a strong influence on oxidative stability of hempseed oil.

Acknowledgment
Authors thank DUO AG Ltd. and especially the director Gatis Poīs for donation of hempseed oil and other plant materials. The studies were financially supported by Riga Technical University within the project “Implementation of innovative preparation technologies of concentrates of avenanthramides in manufacturing of biologically active products”.

References
INFLUENCE OF DIFFERENT SELENIUM CONCENTRATIONS ON THE PROTEIN AND STARCH CONTENT IN RYE MALT

Kristina Antonenko¹, Viesturs Kreicbergs¹, Anda Linina¹, Daiga Kunkulbega²

¹ Department of Chemistry, Faculty of Food Technology, Latvia University of Agriculture, Liela Street 2, Jelgava, Latvia, e-mail: teknologs.lc.97@gmail.com
² Department of Food Technology, Faculty of Food Technology, Latvia University of Agriculture, Liela Street 2, Jelgava, Latvia

Abstract

The research object was rye malt. Experiments were carried out at the Faculty of Food Technology of the Latvia University of Agriculture. The main aim of this study was to investigate the influence of different selenium concentrations on the content of protein and starch in rye malt. The protein, starch, also hectolitre mass were tested by Grains Analyzer Infratec 1241 (Sweden), the Falling number was analysed using Hagberg-Perten method, according the ISO 3093:2009. Rye grain of 95% viability were soaked and germinated at temperature +6±2 °C for 3 days, using sodium selenate Na₂SeO₄ solutions (Se concentration in solution 3 mg L⁻¹, 5 mg L⁻¹, 10 mg L⁻¹), dried in the drier for 24 hours at temperature from 70 to 112 °C. The germination of grain with deionised water served as a control. The obtained results showed that the increase of selenium concentration in solution significantly decreases hectolitre mass (from 60.6 to 59.3 kg hL⁻¹), the protein (from 10.28 to 10.02 mg 100 g⁻¹), starch (from 68.7 to 66.9 mg 100 g⁻¹) content and falling number (from 90.5 to 81.0 s) in rye malt comparing with control sample.

Keywords: rye malt, selenium, protein, starch.

Introduction

During recent years significant progress has been made in the understanding of the processes of uptake and pathways of various chemical elements in plants (Shtangleeva et al., 2011; Kranner, Colville 2011). Selenium (Se) is not an essential element for plants; it is an essential micronutrient for both humans and animals. More than 20 different selenoproteins have been characterized, including glutathione peroxidases and thioredoxinreductase, which are involved in controlling tissue concentrations of highly reactive oxygen-containing metabolites. The consumption of food provides the principal route of Se intake for the general population. The Food and Agriculture Organization of the United Nations (FAO, 2001) recommends a daily Se-allowance for humans of between 6 and 42 μg day⁻¹ depending on age and gender (Bitterli et al., 2010). Average Se concentration in cereals amounts 0.024 mg kg⁻¹ of grain dry matter, ranging from 0.006 to 0.122 mg kg⁻¹, for barley 0.06 mg kg⁻¹, for wheat 0.011 mg kg⁻¹ on the average (Düma, 2006). Plant Se accumulation can vary more than two orders of magnitudes at a given soil Se concentration for a specific form of soil Se (i.e. “native” soil Se, Se added as selenate, or as selenite) among different plant taxa. Differences observed in the transfer of Se from soil into plants appear to result primarily from differences in the solubility of Se species in soil and only to a minor degree from differences in plant uptake efficiency among these species (Shtangleeva et al., 2011; Bitterli et al., 2010).

Plant roots can take up Se in form of selenate, selenite and also organo-Se compounds, e.g., selenocysteine and selenomethionine. Selenium is not available for plant uptake in the form of colloidal elemental Se or metal selenides (Kranner, Colville 2011; Bitterli et al., 2010).

It is important to remember that under ordinary conditions, each plant part may have its own characteristic concentrations of elements; therefore, comparisons of element concentrations in plants may not be referred to the plant as a whole but should refer to the same plant parts (e.g. roots, or leaves, or seeds). Lastly, may assume that not only concentration of one or another element in a particular plant part of any two plant species growing in the same environment may differ significantly but relations between elements in the plants may also be different (Shtangleeva et al., 2011).

Rye (Secale cereale L.) is an important source of whole grain foods in Eastern and Northern European diets (Katina et al., 2007). Rye malt is a natural food product produced by germinating rye grains (Luoto et al., 2012; Siwela et al., 2010). Germinated cereals/pseudocereal or sprouts are believed to have a greater nutritive and physiological value than cereal and pseudocereal grains and their products (Donkor et al., 2012). During germination (i.e. hydrothermal treatment in ambient conditions) the biosynthetic potential of grains is exploited and a number of hydrolytic enzymes are synthesised. The reactions in germinating grain lead to structural modification and the synthesis of new compounds, some of which have high bioactivity and can increase the nutritional value and stability of the grains (Kaukovirta-Norja et al., 2004; Gomand et al., 2011).

Plant derived foods provide an important source of proteins and dietary minerals. This is especially true in developing countries where plant foods are a predominant portion of the diet. The concentrations of some minerals, especially iron, zinc, iodine, and selenium, are inherently low in plants as opposed to animal derived foods. As a result, more than 3 billion people worldwide suffer from micronutrient malnutrition (Waters, Sankaran, 2011).
Protein content shows grain suitability for processing. The temperatures and water stress occurring during grain filling period affects changes in rye protein aggregation. Usually in whole grain rye flour contains 8–13% proteins. The starch composition of cereals plays a major part in the digestibility and bread-making quality of flour. Generally in whole grain rye flour contains 56–70% starch.

Hectolitre mass (HM) is often used as an index of milling quality. The HM can be used as a silo management tool to optimize the storage space in the silo. The moisture content, weathering, kernel size, density, and packing factors affect hectolitre mass. (Zarina, 2012). The minimum hectolitre mass requirement for intervention of rye in the European Union (EU) is 68.0 kg hL⁻¹.

Hagberg falling number (hereinafter falling number) is one of the most important grain quality indices of rye. The Hagberg falling number is an indicator of α-amylase activity and a measurement of how far the break-down of starch has progressed in the kernel through enzymatic activities. It is expressed in seconds (s) and generally provides a measure of α-amylase enzyme activity in the grain. A high falling number indicates minimal activity, whereas a low falling number indicates more substantial enzyme activity (Buchanon, Nicholas 1980). Alpha-amylase activity depends on weather conditions, especially precipitation. Under rainy conditions, the grains of wheat germinate in the ear either before or at harvest-ripeness, known as sprouting in the ear (Kunkulberga et al., 2007).

There is match investigation about selenium uptake, accumulation, biofortification, translocation, enrichment, toxicity, and tolerance in higher-plants (Shtangeeva et al., 2011; Kraner, Colville 2011; Bitterli, 2010;Waters, Sankaran, 2011), but information about selenium influence on the content of starch, protein, hectolitre mass and falling number in rye malt is scarce. The aim of this research was to investigate the influence of different selenium concentrations on the protein and starch content, grains hectolitre mass and falling number in rye malt.

Materials and Methods

Plant material

The object of the research was rye grain (variety ‘Kaupo’) from Ltd. Naukšēni (Latvia) harvested in 2011. Rye grain were soaked and germinated at temperature +6±2 °C for 3 days, using sodium selenate Na₂SeO₃ solutions (Se concentration in solution was 3 mg L⁻¹, 5 mg L⁻¹and 10 mg L⁻¹). The germination of grain with deionised water served as a control. After germination, all sprouts were dried for 24 h at a temperature from 70 to 112 °C; then they were grounded.

Determination of protein content, starch and hectolitre mass

The content of protein, starch and hectolitre mass of rye malt grains were determined by a near-infrared spectroscopy Grain Analyzer Infratec 1241. The contents of protein and starch where calculated based on grain dry matter (dm).

Determination of Falling number

The Hagberg falling number – α-amylase activity – was measured by the Hagberg-Perten method using a Perten Instruments (Sweden) „Falling number 1500” assessed to ISO 3093:2009. The Falling Number laboratory measurements with some modifications are conducted according to a standardized method aimed at measuring the viscosity of a mixture of 6g milled wheat with 1 g milled rye malt and 25 mL water placed in a bath at 100 °C (Gaaloul et al., 2011).

Mathematical data processing

The results were processed by mathematical and statistical methods. The statistical analyses of data were carried out using Microsoft Excel for Windows 7.0 (Microsoft Corporation, Redmond, WA). Mean value, standard deviations and significant value were calculated. p-values<0.05 were regarded as significant.

Results and Discussion

Hectolitre mass

The influences of different selenium concentrations on the hectolitre mass of rye malt are present in Figure 1.

Figure1. The influence of different selenium concentrations on hectolitre mass of rye malt

Analyzing obtained results we can see that significantly changes of rye malt hectolitre mass depends on selenium concentration in solution (p<0.05). The relative decreases for 2.2% of hectolitre mass in rye can be observed at selenium concentration in solution 10 mg L⁻¹ (p=0.00).

Protein content

The obtained results have shown that content of protein in rye malt changes significantly (p<0.05) using for germination solutions with different selenium concentrations. These results are shown in Figure 2. The content of protein in rye malt significantly decreases for 2.5% comparing with control sample using solution where selenium concentration was 5 and 10 mg L⁻¹ (p=0.00). Accordingly, if the concentration of selenium in solution was 3 mg L⁻¹ the decrease was 2% (p=0.001). The decrease of protein and starch content could be reason for the changes of hectolitre
mass and it shows the increase of proteolytic enzymes activity in rye malt.

![Graph showing the influence of different selenium concentrations on the content of protein in rye malt.](image)

**Figure 2. The influence of different selenium concentrations on the content of protein in rye malt**

The results of research relate to Rakcejeva (2007) statement that the proteins were split by proteolytic enzymes, as a result of which the total protein content decreased.

**Starch content**

Starch is the major carbohydrate of ryes. The starch content is limited mainly to the endosperm, and contents between 57.1 and 65.6 g 100 g⁻¹ of dry matter (dm) are reported in rye grains (Hansen et al., 2004). Starch consists of a mixture of amylose (AM) and amylopectin (AP). Typical levels of AM and AP in cereal starches are 22–28% and 72–78% (Gomand et al., 2011). The changes of starch content in rye malt using solutions with different selenium concentrations are shown in Figure 3.

![Graph showing the influence of different selenium concentrations on the content of starch in rye malt.](image)

**Figure 3. The influence of different selenium concentrations on the content of starch in rye malt**

Significant differences in starch content were found in two analyzed samples (p<0.05) (Fig. 3). The obtained results showed that content of starch in rye malt depend on the selenium concentration. It decreases with increasing selenium concentration in solution. At selenium concentration 5 and 10 mg L⁻¹ the content of starch in rye malt relative decreases for 1.9% and 2.6% compared with control sample, but at selenium concentration in solution 3 mg L⁻¹ these changes were not so significant (p=0.240). These changes can be explained that the different selenium concentration affect the activity of amylolytic enzymes as a result of which the total starch content decreased in analyzed samples.

**Hagberg Falling number**

The potential activity of α-amylase in rye flour characterized Hagberg Falling Number (FN). The Hagberg Falling Number is an internationally recognized measure that allows the indirect determination of α-amylase activity. FN is the major quality attribute of rye grain. FN low values reflect high α-amylase levels causing the loaves of bread to be discoloured, sticky and of poor resilience and texture (Gaaloul et al., 2011). If the rye grain is harvested during the optimum time and not sprout-damaged normally has a FN in the range of 150 s, when weather conditions was dry and sunny the FN are 300 s and higher. The minimum FN requirement for intervention of rye in the EU was 100 s (Hansen et al., 2004).

![Graph showing the influence of different selenium concentrations on the falling number of rye malt.](image)

**Figure 4. The influence of different selenium concentrations on the falling number of rye malt**

The influence of different selenium concentrations on the falling number of rye malt is shown in Figure 4. The obtained results have shown that all analyzed Se concentrations significantly promoted the decrease of rye malt falling number and the highest decreases was observed when selenium concentration was 10 mg L⁻¹ (p<0.05). In this case the falling number of rye malt decreases for 10.5% comparing with control sample. Results of present research show the different selenium concentration positive affects the activity of amylolytic enzymes and relative decreases the falling number.

**Conclusions**

The different selenium concentration in solutions affects the composition and of rye malt. The most significant change in rye malt was observed at selenium concentration in solution 10 mg L⁻¹. The relative content of protein and starch decreases for 2.5% and 2.6% compared with control sample it gives evidence about that degradation. The degradation of protein and starch affects the hectolitre mass, the relative decreases of hectolitre mass was 2.2% at selenium concentration in solution 10 mg L⁻¹. The Hagberg Falling Number decreases for 10.5% (selenium contration in solution 10 mg L⁻¹) that characterized the increase of amylase activity.
Acknowledgment
This research has been prepared within the Ltd. NAUKSENI, Nauksenu reg., “Straumeni”.

References
Abstract

Dietary fibre is an important component of human’s nutrition. It is the common name for all carbohydrate components occurring in foods that are non-digestible in the human small intestine. It is known that deficiency of dietary fibre in food, provoke disturbance of intestinal tract, for example, constipation and that different fibre sources have different composition and quantities of dietary fibre. Therefore the task of research was to investigate the content of total, soluble and insoluble dietary fibre in different fibre sources such as wheat and rye bran, defatted flaxseeds, chicory and Jerusalem artichoke powder.

The results of research showed that total dietary fibre content in different fibre sources ranged between 41.76% and 59.42%, where the lowest TDF content was determined for wheat bran and the highest for Jerusalem artichoke powder. The content of TDF in defatted flaxseeds and Jerusalem artichoke powder was significantly higher comparing with other samples (p<0.05). Whereas dried chicory and Jerusalem artichoke powder were important sources of soluble dietary fibre, which significantly differed from other analysed samples (p<0.05). The significant part of SDF content made inulin, which contain in dried chicory was 94.3% of total SDF and in Jerusalem artichoke powder – 97.7%. The significantly higher amount of IDF was determined in wheat and rye bran, defatted flaxseeds (p<0.05) comparing with dried chicory and Jerusalem artichoke powder.

Keywords: bran, flaxseeds, chicory, Jerusalem artichoke, soluble and insoluble fibre.

Introduction

The food industry is facing the challenge of developing new food products with special health – enhancing characteristics, since the beneficial effects of healthy diet on the quality of life are widely recognized. These functional materials come from a wide variety of plant sources which provide important nutraceutical components that may be used in food systems (Lee et al., 2004). Dietary fibre is an important component of human’s nutrition. These health benefits include reduction in bowel transit time (Gear et al., 1981; Brennan, Cleary, 2005), prevention of constipation (Odes et al., 1993), reduction in risk of colorectal cancer in vitro (Rowland, 1995; Verghese et al., 2002), production of short chain fatty acids (Wasan, Goodland, 1996; Scharlau et al., 2009) and promotion of the growth of beneficial gut micro flora (Brennan, Cleary, 2005; Bosaeus, 2004). Fibre is naturally present in cereals, fruits, pulses, vegetables; it can be produced from sources that might otherwise be considered waste products. For example, soy hulls, defatted flaxseeds, oat hulls, spent brewer’s grain and waste portions of fruits and vegetables (Katz, 1997).

Dietary fibre is the common name for all carbohydrate components occurring in foods that are non-digestible in the human small intestine. These components include non-starch polysaccharides, resistant starch, and resistant oligosaccharides with three or more monomeric units, and other non-digestible, but quantitatively minor, components when naturally associated with dietary fibre polysaccharides, especially lignin. Dietary fibre components consist of two major classes: (1) water soluble polymers (SDF), such as pectins and gums, and (2) water insoluble materials (IDF), in which cellulose, hemicelluloses and lignin are included (Lebesi, Tzia, 2011). The Institute of Medicine (USA) recommends that children and adults consume 14 g of fibre for every 1 000 calories of food they eat each day. That means a person who eats 2 500 calories each day should get at least 35 g of fibre daily, while a person who eats 1 700 calories each day needs somewhat less fibre – about 24 g. Here are general fibre intake recommendations for different age groups and genders. These recommendations are based on the average daily calorie intake for people in these age and gender groups. Individuals who consume more or fewer calories than this average should adjust their fibre intake accordingly (Institute of Medicine, Food and Nutrition Board, 2005). In 2010 EFSA set European dietary reference values for nutrient intakes, where is written that a daily intake of 25 g of dietary fibre is adequate for normal bowel function in adults. In Latvia man consumes 20.2 g per day, women 15.8 g per day (Joffe et al., 2009). It is less than recommended daily intake of fibre. The part of nutritionists and diet experts suggest that 20–30% of daily fibre intake should come from soluble fibre (Institute of Medicine, Food and Nutrition Board, 2005).

Bran represents the outer parts of grains including the pericarp and surrounding cuticle, the testa and the aleurone layer. Depending on the milling process, commercial bran preparations also contain variable amounts of starchy endosperm and germ (Kamal-Eldin et al., 2009). Wheat bran is widely commercially available, are well characterised with respect to their composition and properties, especially with regard to their fibre components (Luhaloo et al., 1998). Wheat bran is the best known source of insoluble dietary fibre which helps to prevent and control bowel problems and to lower cancer risk (Verma, Banerjee, 2010). Whereas rye consumption inhibits breast and colon tumour growth in animal models, lower glucose responses in diabetics, and lowers the risks of death from coronary heart diseases (Verma, Banerjee, 2010).
Defatted flaxseeds contain circa 30% dietary fibres of which one third is viscous and the majority of the flaxseed water-extractable dietary fibre (the mucilage) belongs to heterogenic polysaccharides. Viscous dietary fibre has been shown to induce an increased sensation and lowered energy intake at the following meals (Archer et al., 2004). Defatted flaxseeds are commercially available as the product rich in fibre, proteins, and polyunsaturated fatty acids. This dietary source is from oil pressing, using cold pressing method. Defatted flaxseeds are dietary fibre source, which are primarily recognised as a rich source of alpha linolenic acid and plant lignin and have as such been proposed to play a role in cardiovascular disease prevention (Bloedon, Szapary, 2004).

Chicory (Cichorium intybus) is one of the earliest known and most widely used raw materials for the manufacture of coffee substitutes (Pazola, 1987). The major component of chicory root is inulin which is a polymer of fructose with (2-1) glycoside linkages. Inulin is soluble in water and not hydrolyzed by human digestive enzymes, it is expected to behave like a soluble (Gibson et al., 1995). Inulin is used in the food industry not only as a source of dietary fibre (Flamm et al., 2001), but also as a functional food ingredient since it affects biochemical and physiological processes resulting in better health and reduction in the risk of many diseases in humans beings (Kaur, Gupta, 2002). Jerusalem artichoke (Helianthus tuberosus L.) is cultivated mainly for use as green or ensiled forage, as a cover crop in marginal areas and to produce sugars (especially fructose) and fructans (inulin), which are used as food or for various chemical, electronic and pharmaceutical applications (Bosticco et al., 1989; Maijer, Mathijssen, 1991). Jerusalem artichoke is one of the most important candidates for use as a raw material for the industrial production of biological fructose and inulin. It is a particularly interesting and suitable crop, for southern European countries and especially in low-requrement environments (Paolini et al., 1998; D’Egidio et al., 1998).

It is known that deficiency of dietary fibre in food, provoke disturbance of intestinal tract, for example, constipation and that different fibre sources have different composition and quantities of dietary fibre. Therefore the task of research was to investigate the content of total, soluble and insoluble dietary fibre in different fibre sources such as wheat and rye bran, defatted flaxseeds, chicory and Jerusalem artichoke powder.

### Materials and Methods

The research was performed at the Food technology laboratory of the Department of Food Technology of Latvia University of Agriculture and at the laboratory of Microbiology and Biotechnology Department of the Faculty of Biology of Latvia University.

 Materials used in the research were wheat bran (Farm “Paukulnieki”, Latvia), rye bran (Farm “Paukulnieki”, Latvia), defatted flaxseeds with fat content less than 10% (“Iecavnieks and Co”, Latvia), dried chicory (“RA5”, Latvia) and Jerusalem artichoke (“Herbe”, Latvia). Wheat and rye bran as well dried chicory were milled for analysis.

The content of total, soluble and insoluble dietary fibre was determined by AOAC Official Method: 991.43; Total, Soluble and Insoluble Dietary Fiber in Foods Enzymatic-Gravimetric Method (Phosphate buffer) and using the FOSS Analytical Fibertec E 1023 System. The content of inulin was determined by AOAC Official Method 999.03 and by AACC Official Method 32.32.

The analyses were performed in triplicate. The differences in the content of total, soluble and insoluble dietary fibre were analyzed using the analysis of variance (ANOVA). t-test was applied to compare the mean values, and p-value at 0.05 was used to determine the significant differences.

### Results and Discussion

The total dietary fibre (TDF) content in different fibre sources such as wheat and rye bran, defatted flaxseeds, dried chicory and Jerusalem artichoke powder is given in Table 1. The obtained results were compared with literature data for analysis.

#### Table 1

**Comparison of total dietary fibre content in analyzed samples with literature data, %**

<table>
<thead>
<tr>
<th>Samples</th>
<th>Total dietary fibre (TDF)</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat bran</td>
<td>41.76±1.54</td>
<td>Kamal-Eldin et al., 2009</td>
</tr>
<tr>
<td>Rye bran</td>
<td>42.28±0.98</td>
<td>Kamal-Eldin et al., 2009</td>
</tr>
<tr>
<td>Defatted flaxseeds</td>
<td>57.41±1.03</td>
<td>Schakel et al., 2001</td>
</tr>
<tr>
<td>Dried chicory</td>
<td>46.10±1.09</td>
<td>Schittenhelm, 1996</td>
</tr>
<tr>
<td>Jerusalem artichoke powder</td>
<td>59.42±1.21</td>
<td>Gedrovica, 2012</td>
</tr>
</tbody>
</table>

*a Total dietary fibre content with inulin  
*b Total dietary fibre content in raw flaxseeds  
*c There is indicated only inulin

Total dietary fibre (TDF) content in different fibre sources ranged between 41.76% and 59.42%, where the lowest TDF content was determined for wheat bran and the highest for Jerusalem artichoke powder. Comparing the research data with literature it was established that obtained results were close to data given in literature except defatted flaxseeds and dried chicory. The differences of TDF content in flaxseeds could be explained with various used materials: raw flaxseeds (data of literature) and defatted flaxseeds (data of research). The literature data of TDF content in defatted flaxseeds was not available. The differences of
TDF in dried chicory could be chained with various factors such as chicory sort, growing conditions, season, and region as well treatment technology. The results of dispersion analysis established that there were no significant differences of TDF content among wheat and rye bran as well dried chicory (p>0.05). Whereas the content of TDF in defatted flaxseeds and Jerusalem artichoke powder was significantly higher comparing with other samples (p<0.05). Evaluating the dietary fibre content there is important the amount of soluble and insoluble dietary fibre in product. Therefore the content of soluble and insoluble dietary fibre in analysed fibre sources is shown in Figure 1.

![Figure 1. Content of soluble and insoluble dietary fibre in different fibre sources](image)

The obtained results showed that dried chicory and Jerusalem artichoke powder were important sources of soluble dietary fibre (SDF), which significantly differed from other analysed samples (p<0.05). The significant part of SDF content made inulin, which contain in dried chicory was 94.3% of total SDF and in Jerusalem artichoke powder – 97.7%. Due inulin contain in dried chicory and Jerusalem artichoke powder the both fibre sources have been attempted in different products attributed to their health benefits. Inulin possesses the typical properties of fibre – reduction of transit time in bowel, decreasing of pH in colon, beneficial effect on blood indices (Roberfroid, 1993). The SDF content in rye bran confirm the conclusions of Åman et al. (1997) research that rye fibre contains soluble fibre 11–12%, i.e., in the form of arabinoxylan (9%) and β-glucan (2–3%). Evaluating the content of insoluble dietary fibre (IDF) in analysed samples there was determined significantly higher amount of IDF in wheat and rye bran, defatted flaxseeds (p<0.05) comparing with dried chicory and Jerusalem artichoke powder. The obtained results correspond to Verma and Banerjee (2010) investigations that wheat bran is the best known source of insoluble dietary fibre. The present study showed that different fibre sources had various amount of soluble and insoluble dietary fibre. Therefore it is important to mix different fibre sources for producing new functional products with advisable soluble and insoluble dietary fibre correlation.

**Conclusions**

Total dietary fibre (TDF) content in different fibre sources ranged between 41.76% and 59.42%, where the lowest TDF content was determined for wheat bran and the highest for Jerusalem artichoke powder. The content of TDF in defatted flaxseeds and Jerusalem artichoke powder was significantly higher comparing with other samples (p<0.05). Whereas dried chicory and Jerusalem artichoke powder were important sources of soluble dietary fibre, which significantly differed from other analysed samples (p<0.05). The significant part of SDF content made inulin, which contain in dried chicory was 94.3% of total SDF and in Jerusalem artichoke powder – 97.7%. The significantly higher amount of IDF was determined in wheat and rye bran, defatted flaxseeds (p<0.05) comparing with dried chicory and Jerusalem artichoke powder.

**References**


DETERMINATION OF THE OPTIMAL RATIO OF RECIPE INGREDIENTS IN THE PROCESS OF DESIGNING CONFECTIONERY PRODUCTS

Polina Smolikhina, Evgeniia Muratova

Tambov State Technical University, Tambov, Sovetskaya st., 106, Russian Federation, e-mail: topt@topt.tstu.ru

Abstract

The aim of this study was to develop new recipes of confectionery products for functional purposes in accordance with predetermined criteria of optimization.

The paper formulates the objective of recipe optimization relating to the class of multicriteria nonlinear programming problems, and shows the algorithm developed for its solution. As an example, the article gives the formulation and solution of the problem that involves designing candy recipes with mixed jelly-whipped body. The choice of ratio between layers of combined body enriched with functional ingredients was carried out according to four criteria (energy value, carotenoids, dietary fiber, and the cost price of the finished product) under the given constraints on technological and consumer product characteristics.

The solution of the multicriteria problem identified a number of effective points belonging to the area of Pareto. The final choice of possible options was made on the basis of the ratio “price–quality” tailored to meet the daily requirements of micronutrients and organoleptic evaluation of the product.

Adjustment of the layers provides a wide range of jelly-whipped candies with original organoleptic characteristics and a given set of micronutrients that can be recommended for different categories of the population. Varying the composition of recipe mixtures from possible set of raw ingredients, enriching them with micronutrients, can influence on the physiological effect of finished products.

The proposed approach can be recommended for recipe designs of complex multicomponent food systems with specified set of characteristics in terms of multiple alternatives of ingredient composition and interchangeability of raw materials.

Keywords: recipe calculations, multicriteria optimization.

Introduction

Increasing the competition in raw materials and food markets, the growing need for products of functional purposes, a variety of raw ingredients in the market foreground issues on the design of new recipes and their corrections depending on fluctuations in raw material parameters.

According to modern ideas the concept of „food design“ includes the development of models describing the steps for creating products of specified quality and representing mathematical relationships which reflect different functional connections between technological, economic and other parameters of raw ingredients, desired characteristics of finished products (objective function) and a number of constraints arising from the requirements of normative documents (Киселев, Першина, 2009; Муратова, Толстыkh et al., 2010).

One of the important objectives in the design of new types of functional foods for various categories of the population is to provide the optimal set of recipe ingredients and ratios in multicomponent food systems. The difficulty of solving this problem is related to the fact that:

- raw material base of the modern food industry employs thousands of ingredients that differ in functional, technological, physical, chemical and organoleptic characteristics;
- semi-finished products should have a given complex of technological properties;
- finished products must comply with the balanced nutrition formula, respond to medical and biological requirements, have high consumer properties and be affordable to the public.

The need to consider a variety of recipes, to take into account properties of semi-finished and finished products according to the criteria and limits for each stage of designing a new product requires the involvement of mathematical support, software algorithms and the use of automated systems as essential tools for solving the problem of determining the optimal ratio of recipe ingredients (Muratova et al., 2010; Дворецкий et al., 2012).

Materials and Methods

To maximize the use of micronutrient complex of different chemical nature that is a part of functional additives of plant origin, and to create a broad product line of candies a combination of candy mass that have different composition and colloid-chemical properties can be used. As functional additives we used powdered semi-finished products prepared from local plant raw materials with different dispersion from 0.14 to 0.5 mm. Pumpkin and carrot powders containing vitamins of groups A, B, C, E, carotenoids and other vitamin-like substances, dietary fibre, macro- and micronutrients (potassium, calcium, iron, magnesium) have been received through combined convective-vacuum impulse drying that allows to keep all biologically valuable substances included in raw.

For enriching products with functional additives we used whipped cream and jelly candy mass with gelatinous consistency and low, compared to other confectionery, energy value. The composition of ingredients for preparing the whipped mass included sugar, agar, syrup, egg white, carrot powder, and citric acid. The composition of jelly mass included sugar, syrup, pectin, pumpkin powder, citric acid, sodium citrate.
When creating recipes we considered the possibility of chemical interaction between ingredients and chose combinations and methods of application ensuring their maximum safety during production and storage as well their high bioavailability.

During the research we applied commonly used and special research methods for studying the properties of raw materials, semi-finished and finished products. The experimental data were processed by methods of mathematical statistics using software packages MS Office Excel, MathLab 7.1. When designing formulations used the method of multi-objective optimization using LPr sequences (Sobol, Statnikov, 2006).

It is necessary to consider the problem of designing confectionary recipes based on combining jelly and whipped candy mass with gelatinous consistency and the same moisture content. As the functional ingredient we used pumpkin powder (3 wt.%) in the jelly mass and carrot powder (10 wt.%) in the whipped mass (Muratowa, Smoliikhina, 2013). The set of alternative raw ingredients and main production stages of candies with combined whipped-jelly bodies for functional purposes are well known. By varying the composition of recipe mixtures and enriching them with micronutrients we can achieve certain physiological effects. For designing multicomponent food products object-oriented approach is offered to use. A distinctive feature of the object-oriented approach to recipe design of multicomponent food systems is presenting the recipe in a hierarchical structure.

Results and Discussion

It is important to determine the recipe $x = (x_1, x_2, ..., x_n)$ of the finished product in which the basic consumer characteristics (food, biological and energy value, etc.) and cost price of the finished product $F(x) = (f_1(x), f_2(x), ..., f_m(x))$ reach the optimal values, and other consumer characteristics (microbiological indicators, shelf life, safety performance) $g_j(x), j = 1, 2, ..., m$ satisfy the requirements of the technical specification for developing new food recipes, i.e. $g_j(x) \leq 0, j = 1, 2, ..., m$.

The problem of mathematical formulation of optimal recipe design is as follows:

$$x^* = \text{arg min}_{x \in X} \{F(x)\}$$  \hspace{1cm} (1)

Restrictions on other consumer characteristics of the finished product:

$$g_j(x) \leq 0, j = 1, 2, ..., m$$  \hspace{1cm} (2)

and the ratio of raw ingredients and semi-finished products:

$$x_i \leq x_j, i = 1, n$$  \hspace{1cm} (3)

The formulated problem belongs to the class of multiobjective problems in nonlinear programming. The problem was solved on the basis of the most-informative presentation of the searched area for compromise solutions by points filling the area $X$ according to the method of I.M. Sobol. Algorithm for solving this problem is presented in the paper (Dvoróchuk et al., 2012).

Solving the problem of optimal recipe design of jelly-whipped candies enriched with functional ingredients (1)-(3) supposes using the applied criteria: carotenoids, $f_1(y, x)$; dietary fibre content, $f_2(y, x)$; energy value $f_3(y, x)$, cost price of the finished product $f_4(y, x)$.

Characteristics of the whipped and jelly layers are shown in Table 1.

Objective functions are calculated by the formula:

$$f_1(x) = \sum_{j=1}^{2} c_j x_j, i = 1, 4$$  \hspace{1cm} (4)

where $x_j, j = 1, 2$ – percentage of the whipped and jelly layer, respectively; $c_1, j = 1, 2$ – energy value of constituent layers, kcal; $c_2, j = 1, 2$ – carotenoids, mg; $c_3, j = 1, 2$ – dietary fiber, g; $c_4, j = 1, 2$ – cost price, rubles.

Table 1

<table>
<thead>
<tr>
<th>Characteristics of jelly and whipped layers</th>
<th>Range of admissible values, wt.%</th>
<th>Energy, kcal</th>
<th>Carotenoids, mg</th>
<th>Dietary Fiber, g</th>
<th>Cost, rubles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jelly layer, 20.1–10.0–90.00</td>
<td>20.1–321.0</td>
<td>0.01–0.56</td>
<td>0.40–6.60</td>
<td>6.80–58.90</td>
<td></td>
</tr>
<tr>
<td>Whipped layer, 28.4–10.0–90.00</td>
<td>28.4–463.0</td>
<td>0.50–7.50</td>
<td>0.02–11.60</td>
<td>0.38–103.50</td>
<td></td>
</tr>
</tbody>
</table>

As a result of solving the multicriteria problem (1)-(3) many effective points $(y, x)$ of Pareto area (F) were built, which may be recommended for different recipes of jelly-whipped candies with high nutritional value taking into account consumer preferences:

1) $y = 2.3, F = (2.6; 11.76; 363.6; 0.8029)$ – low energy value and high content of dietary fibre – can be recommended for the diet;

2) $y = 1.0, F = (4.09; 8.22; 380.0; 91.1)$ – balanced composition and average cost – for mass consumption;

3) $y = 0.4, F = (5.38; 4.6; 386.0; 100.35)$ – high energy value and high antioxidant content – can be recommended for sports nutrition.

The final choice of the above mentioned options was made on the basis of the ratio “price–quality” tailored to meet the daily requirement of micronutrients and organoleptic evaluation of the product. Adjustment of the layers provides a wide range of jelly-whipped candies with original organoleptic characteristics and a given set of micronutrients (Figs. 1 and 2).
Taking into account the established and well-known rules of biologically active substances norm losses during technological processing and storage we calculated food and energy value of jelly-whipped candies with the addition of vegetable powders and different ratios of the body layers (Table 2).

For the calculation of multi component food recipes we used an automated system „Multifaz“ based on the object-oriented approach (registration number 2011616793) and developed by the staff of Tambov State Technical University.

A distinctive feature of the object-oriented approach to the recipe design of multicomponent food systems is presenting the recipe as a hierarchical structure (Fig. 3).

Each of the top in the hierarchical structure is an object (the finished product, semi-finished product, raw materials). Every hierarchy level corresponds to a specific stage of manufacturing the food product and may have its own individual number of tops positioned lower in the hierarchy. Figure 3 shows a three-level hierarchy of calculating recipes, where the first index – the level number, the second index – the number of recipe component mixture. If on one or another recipe level several semi-finished products are used, their first index becomes composite and is denoted as a list \((i, j)\), where \(i\) – the level number; \(j\) – the serial number of the semi-finished product on the \(i\) level. This composite index is used below in the hierarchy (shown by the dashed arrow).

The algorithm for calculating multiphase food product recipe begins with the calculation of the final level in the longest branch of the hierarchical structure of the calculation. The initial data for calculation of the last level are: the consumption on downloading all kinds of raw materials and semi-finished products in kind; the loss of dry substances; the predetermined amount of finished products equal to 1 t.

Functional diagram of the automated information system is presented in Figure 4 (Муратова, Толстых, 2010). The system consists of information database

---

**Table 2**

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Food value</th>
<th>With the addition of vegetable powders and the ratio of the body layers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>30:70</td>
</tr>
<tr>
<td>Energy value per 100g of candies, kcal (kJ)</td>
<td>400.0 (1676.0)</td>
<td>386.0 (1617.3)</td>
</tr>
<tr>
<td>Proteins, g</td>
<td>1.80</td>
<td>2.08</td>
</tr>
<tr>
<td>Fat, g</td>
<td>15.18</td>
<td>17.71</td>
</tr>
<tr>
<td>Carbohydrates, g</td>
<td>62.99</td>
<td>54.29</td>
</tr>
<tr>
<td>BAS Per 100g (satisfying the norm of physiological needs, %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dietary fibre, g</td>
<td>0.4 (7.7)</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>(21.3)</td>
<td></td>
</tr>
<tr>
<td>Vitamin C, mg</td>
<td>0.4 (0.54)</td>
<td>12.8</td>
</tr>
<tr>
<td></td>
<td>(18.2)</td>
<td></td>
</tr>
<tr>
<td>Carotenoids, mg</td>
<td>0.02</td>
<td>5.3</td>
</tr>
</tbody>
</table>
including recipe components and semi-finished products, calculation and optimization modules. Information module is a collection of data for recipe components and semi-finished products.

![Figure 4. Functional diagram of the system „Multifaz”](image)

The calculation module is used for selecting and recording components into the database information module, making calculations of single-phase and multiphase recipes, as well as food and energy value. The optimization module performs the selection of recipe components according to food and energy value in terms of restrictions on the cost price (Figure 5)

![Figure 5. Diagram of program modes for the calculation of single-phase recipe](image)

To construct the recipe hierarchy from the list of raw semi-finished components it is necessary to select semi-finished products from which the product is made and set the amount for the load. Then each recipe of semi-finished products is prepared with indicating solids content and the possibility of adding new recipe components to the list. Next the loading characteristic is set for each component of the raw material. The general hierarchy view of the wafers recipe for functional purposes is shown in Figure 6.

Pressing the button „Calculation” we get raw material consumption for cooking semi-finished products in accordance with this recipe, as well as a summary recipe of the designed product.

![Figure 6. General view of the recipe tree of products for functional purposes](image)

Calculation module of the automated system “Multifaz” has been tested and used in the educational process during the study of the discipline „Design of combined food” and during performing the final qualification work of bachelors, specialists and masters. The development of software module for optimizing recipes on food and energy value is now in progress.

Conclusions

Computer-aided recipe design noticeably allows to accelerate the calculation and optimization of complex multi component food products, to create products with predetermined chemical composition, nutritional value and functional orientation, to respond quickly to changes in the properties and types of raw ingredients.

Acknowledgment

The authors thank Doctor of Engineering, Professor S.I. Dvoretskiy for advice on the use of mathematical modeling for recipe design and optimization of complex multicomponent food products, Ph. D. Associate Professor S.S. Tolstykh for the development of algorithms and software for the calculation of recipes and Ph. D. E. Yu. Voyakina for the translation of the article.

References


INTERACTION OF SUGAR CONFECTIONERY SHERBET QUALITY PARAMETERS DURING STORAGE TIME IN DIFFERENT PACKAGING

Lija Dukalska, Eva Ungure, Sandra Muizniec-Brasava

Department of Food Technology, Faculty of Food Technology, LatviaUniversity of Agriculture, 2 Liela street, Jelgava, Latvia, e-mail: Lija.Dukalska@llu.lv

Abstract

Essential importance for milk pomade confectionery quality assurance during storage time is alternate design of appropriate packaging technologies and materials, wherewith in this research in order to substantiate scientifically the optional shelf life of sherbet, different packaging materials in air ambiance, modified atmosphere (MAP), and active packaging with oxygen absorbers were used to approve their conformity for providing the main physical parameters and microbiological security during long term storage of investigated sugar confectionery. Shelf life can be extended significantly in conventional polymer packaging with high barrier properties: to 10–12 weeks in air ambiance OPP and Multibarrier 60 HFP material; to 16 weeks in MAP 100% CO₂ and Multibarrier 60 HFP, metallised BOPET/PE and Aluthen material, exceeding neither experts’ accepted hardness of 300 N nor permissible total plate count of microorganisms’ level (TPC g⁻¹≤10⁵). The sherbet hardness in active packaging after 16 weeks of storage in Multibarrier 60 HFP is 261.18±8.32 N, in comparison with MAP 100% CO₂ environment, it is by 11.5% lower, which is valued as positive.

Keywords: confectionery, sherbet, quality, packaging.

Introduction

Nowadays confectionery products are especially popular among children and elderly people, thus the popularity and consumption of these products are increasing (Duran et al., 2009). The European candy’s market is fragmented. Leading market partners are Mars (14.3%), Nestle SA (8.9%), and Cadbury plc (8.6%), the remaining part of market fill small producers – 68.3%. Leading partners in confectionery market of Latvia are Join-Stock Company Laima, Ltd Skrīveru sāldumi, Ltd Pure Chocolate, and SIA Bona Dea. Join-Stock Company Laima is the largest producer of confectionery in Latvia, and at present Laima is the major sweet manufacturer in the Baltic (Laima, 2013).

With development of the choice of sugar confectionery products, most actual becomes the question about the preservation of a shelf-stable quality of the above mentioned products for a longer time, convenient use for consumers as well as opportunity to export the product more successfully (Brown, 2011).

At present in Latvia, confectionery products are sold in two ways – as bulk products which are weighed at the trading place, and as products packed at the manufacturing enterprises in a certain size of commercial packaging. Consistency of the product quality is affected significantly during shelf life by environment that surrounds the product. In oxygen environment, irreversible changes take place in foodstuffs, for instance fats and oils oxidize and become bitter, vitamin content reduces, colour changes, product loses its aromatic substances, and aerobic microorganism growth takes place. A decrease of foodstuff quality in presence of oxygen is also facilitated by increased temperature, moisture, light, especially ultraviolet light. During the course of time, more and more new opportunities are created how to preserve food quality, and scientists suggest new technologies in the packaging industry (Labuza et al., 2004; Subramaniam, 2000; Sucharzewska et al., 2003).

Important factor influencing the confectioneries shelf life is moisture migration from product and its permeation through packaging accordingly affecting the physical-chemical indices of product (Ergun et al., 2010; Willis, 1998). Ergun, Lietha and Hartel indicated that moisture content losses of candies produced on sugar bases have fundamental importance for quality maintenance. Producers should improve packaging technologies to eliminate oxygen and moisture destructive effect.

Nowadays, one of the most perspective methods how to extend the product shelf life is packaging in modified environment – vacuum or modified atmosphere packaging (MAP), where oxygen content is decreased to minimum but the carbon dioxide content is increased allowing to extend the storage time of the product by some days or even several months (Ahvenainen, 2010; Devlieghere, Debevere, 2003; Lagaron, Lopez-Rubio, 2010). Active packaging is an innovative packaging technology, reducing the oxygen content below <0.1%, thus increasing the storage time of foodstuffs (Gibis, Rieblinger, 2011). In order to provide the product quality constancy and extend its shelf life, it is recommended to use packaging materials with high barrier properties as well as to seek for new developments, the latest generation of biomaterials and active packaging technologies. Scientists Londhe, Pal un Raju have investigated the shelf life of Asian sweet stuff – brown peda by packaging interventions, in conventional cardboard boxes, modified atmosphere and vacuum packaging techniques during storage for 40 days at 30±1 °C and concluded that brown peda could be best preserved in vacuum packaging without appreciable quality loss (Londhe et al., 2012). Brown peda is candy with low moisture and high sugar content, highly of same kind like sugar confectionery sherbet.

In this research in order to substantiate scientifically the optional shelf life of sherbet, different packaging materials in air ambiance, modified atmosphere
(MAP), and active packaging with oxygen absorbers were used to approve their conformity for providing the main physical parameters and microbiological security during long term storage of investigated sugar confectionery.

Materials and Methods

The object of the research was milk pomade sweet – sherbet with crunchy peanut chips, produced by stockholder Laima, Latvia. Dimensions of one piece of sherbet in average was 40×40×8 mm, mass 30±1 g. Sherbet was packed by two pieces in a package, the total weight per package – 60±2 g. The size of each bag was 80×120 mm. Samples were packed in air ambience, vacuum packaging, MAP, and in active packaging with oxygen absorbers, stored at room temperature 18.0±3.0 °C, relative air moisture (RH) 40%. Samples were analyzed before packaging and after 2, 4, 6, 8, 10, 12, 14, and 16 weeks of storage.

At present research the most widely used traditional packaging technologies (in air ambience, vacuum and MAP) in conventional packaging materials (Table 1) and innovative (environmentally friendly) materials (Table 2) were studied; also oxygen absorbers in active packaging were applied, and its effect on the quality of sugar confectionery products during the storage time was estimated.

Table 1 Characteristics of conventional packaging materials used in experiments

<table>
<thead>
<tr>
<th>Packaging material</th>
<th>Composition</th>
<th>Thickness, μm</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPP</td>
<td>Single layer, transparent OPP</td>
<td>40±2</td>
</tr>
<tr>
<td>Multibarrier 60 HFP</td>
<td>Transparent laminate, APA/TIE/PA/EVO H/PA/TIE/PE/PE</td>
<td>60±2</td>
</tr>
<tr>
<td>BIALON 50 HFP</td>
<td>Frosty white, laminate, BOPA/PE</td>
<td>50±3</td>
</tr>
<tr>
<td>BIALON 65 HFP</td>
<td>Transparent laminate, BOPA/PE</td>
<td>65±3</td>
</tr>
<tr>
<td>PP</td>
<td>Single layer, transparent PP</td>
<td>40±2</td>
</tr>
<tr>
<td>met.BOPET/PE</td>
<td>Metallised laminate BOPET/ALU/PE</td>
<td>65±2</td>
</tr>
<tr>
<td>Aluther</td>
<td>Metallised laminate PET/ALU/PE</td>
<td>80±2</td>
</tr>
</tbody>
</table>

Packaging in air ambience. At present, the most often commercially applied packaging of sugar confectionery products is air ambience in cardboard boxes, transparent PP bags and metallised PP pouches.

Vacuum packaging. Products were put into initially from polymer film thermally sealed bag; after that, the air was removed or vacuum was created, and then the bag was sealed hermetically (Robertson, 2011).

Modified Atmosphere Packaging. For food packaging, MAP of carbon dioxide CO₂ (E 290) and nitrogen N₂ (E 941) were used, supplied by AGA, Ltd. In experiments, the following gas mixtures were used: 30% CO₂ and 70% N₂; 70% CO₂ and 30% N₂; 100% CO₂. The product was put into initially of different materials made polymer film bags; then, the air was removed from the bags and replaced by gas mixture prepared in gas mixer KM100-2MEM, and the package was hermetically sealed.

Table 2 Characteristics of biodegradable packaging materials used in experiments

<table>
<thead>
<tr>
<th>Packaging material</th>
<th>Composition</th>
<th>Thickness, μm</th>
</tr>
</thead>
<tbody>
<tr>
<td>BIO NVS</td>
<td>Single layer, transparent, cellophane based biodegradable NVS film</td>
<td>25±1</td>
</tr>
<tr>
<td>Ceramis®-PLA</td>
<td>PLA coated with SiOx</td>
<td>60±2</td>
</tr>
<tr>
<td>met. NatureFlex 23NM</td>
<td>Metallised / coating / cellulose film / coating</td>
<td>23±2</td>
</tr>
<tr>
<td>Nativia™ NTSS-30</td>
<td>Transparent BOPLA film</td>
<td>30±2</td>
</tr>
<tr>
<td>Nativia™ NZSS-20</td>
<td>Metallised BOPLA pouch</td>
<td>20±2</td>
</tr>
<tr>
<td>ECOLEAN film</td>
<td>Single layer, white, 40% Ca / 60% PE</td>
<td>78±4</td>
</tr>
<tr>
<td>BIO NVS film</td>
<td>Single layer, transparent, cellophane based NVS</td>
<td>25±1</td>
</tr>
</tbody>
</table>

For reduced oxygen packaging (ROP) creation (O₂ – 0%) in pouches an iron based oxygen scavenger sachets of 100 cc obtained from Mitsubishi Gas Chemical Europe Ageless® were used (Ageless, 2011). The samples were hermetically sealed by MULTIVAC C300 vacuum chamber machine and stored at the room temperature of +21.0±1 °C, (controlled by MINILog Gresinger electronic) and about 40% RH for 12 weeks under day and night conditions. The materials for experiments were selected with different water vapour transmission rate and various thicknesses. To achieve a more active oxygen removal, the active absorber was combined with MAP (100% CO₂)

The following mechanical and physical characteristics were analyzed:

1. The dynamics of gas composition in a hermetically sealed package headspace during the storage time was measured as a percentage of oxygen and carbon dioxide by a gas analyser OXYBABY® V O₂/CO₂.
2. Moisture content accordant at the storage time was determined by using verified balance KERN (Germany) with precision ±0.001g; mass loss calculation (%) – were determined by weighing on the electronic scales.
3. Hardness for freshly manufactured sherbet samples was determined as cutting force (in N) by using
TA-XTplus Texture Analyser. Cutting force was determined for six small sherbet samples from each it piece. A special probe with knife edge for a cut test HDP/BSK blade set with knife was applied. The maximum cutting force (in N) was detected at the deformation rate 10 mm s\(^{-1}\) and distance 10 mm. The samples were cut right through, in order to check whether any different structural characteristics (peanut pieces) were present under the knife inside the product or on the surface. Plotting force (in N) versus storage time (in weeks), the hardness change of sherbet stored in each gas composition in the package as well as for each packaging material was calculated. The maximum cutting force (N) was used as an index for the cut test.

4. For determination of mesophyll aerobic and facultative anaerobic microorganism colony forming unit count (TPC) a standard method was used LVS EN ISO 4833:2003. For determination of yeasts and moulds colony forming units count LVS ISO 21527:2-2008 was used.

5. Statistical analysis. Figures and Tables were developed and calculations were carried out with MS Excel program and SPSS 16 statistics program. The hypotheses were checked with a p value method. Factors were estimated as significant when the p value was <0.05. For interpretation of results it was accepted that α=0.05 with 95% of confidence if not indicated otherwise (Arihipova, Bāliņa, 2006; Bower, 2009).

Results and Discussion

Following the assessment of analysed quality parameters of sugar confectionery product sherbet samples (in 30% CO\(_2\) + 70% N\(_2\), 70% CO\(_2\) + 30% N\(_2\), 100% CO\(_2\)), as optimal packaging environment there was accepted 100% CO\(_2\). In order to find out the effect of the packaging materials on the sherbet, the dynamics of the product hardness during the storage time in air ambience (Fig. 1) and MAP 100% CO\(_2\) (Fig. 2) in different packaging materials are compared. Using a five-point hedonic scale, the hardness level of samples was previously determined when still the product is good for consumption. The initial sherbet hardness have been determined 55.80±2.96 N. Sensory evaluation recognized that the product could be considered usable for consumption up its hardness 300 N. The effect of the packaging material on the hardness during the storage is significantly different (p<0.05). The sample kept in a cardboard box, during two weeks of storage had already reached 300 N. Sherbet packaged in biodegradable packaging materials could be stored 2 to 6 weeks; while in other packaging materials, sherbet could be stored from 6 to 16 weeks. Comparing the effect of packaging technologies on the dynamics of sherbet hardness during the storage, it has been approved that the sherbet storage time in one and the same packaging material is different (Fig. 3). If the recommended shelf life in a cardboard box was 2 weeks, then in Multibarrier 60 HFP it was ranging from 8 to 16 weeks. The shelf life in Multibarrier 60 HFP packaging and 100% CO\(_2\) ambience could be provided to 16 weeks, in addition its hardness was below 300 N. However in the active packaging using oxygen absorbers during the same time its hardness was by 11.5% less; that could be assessed as positive result.

![Figure 1. The dynamics of sherbet hardness during the storage time in air ambience](image1)

![Figure 2. The dynamics of sherbet hardness in MAP (100% CO\(_2\)) during the storage time](image2)
The dynamics of sherbet sample moisture content during the storage time in air ambience, %

<table>
<thead>
<tr>
<th>Sample</th>
<th>0</th>
<th>2</th>
<th>6</th>
<th>8</th>
<th>12</th>
<th>14</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-G</td>
<td>3.40±0.12</td>
<td>2.27±0.10</td>
<td>1.67±0.09</td>
<td>1.63±0.09</td>
<td>1.50±0.09</td>
<td>1.53±0.09</td>
<td>1.31±0.08</td>
</tr>
<tr>
<td>2-G</td>
<td>3.40±0.12</td>
<td>3.05±0.12</td>
<td>2.74±0.11</td>
<td>2.56±0.11</td>
<td>2.55±0.11</td>
<td>2.29±0.10</td>
<td>2.26±0.10</td>
</tr>
<tr>
<td>3-G</td>
<td>3.40±0.12</td>
<td>3.17±0.12</td>
<td>2.95±0.11</td>
<td>2.81±0.11</td>
<td>2.55±0.11</td>
<td>2.40±0.10</td>
<td>2.20±0.10</td>
</tr>
<tr>
<td>4-G</td>
<td>3.40±0.12</td>
<td>2.61±0.11</td>
<td>2.46±0.10</td>
<td>2.31±0.10</td>
<td>2.14±0.10</td>
<td>2.14±0.10</td>
<td>2.03±0.10</td>
</tr>
<tr>
<td>5-G</td>
<td>3.40±0.12</td>
<td>2.82±0.11</td>
<td>2.56±0.11</td>
<td>2.36±0.10</td>
<td>2.19±0.10</td>
<td>2.00±0.10</td>
<td>1.80±0.09</td>
</tr>
<tr>
<td>6-G</td>
<td>3.40±0.12</td>
<td>3.14±0.12</td>
<td>2.95±0.11</td>
<td>2.92±0.11</td>
<td>2.50±0.10</td>
<td>2.28±0.11</td>
<td>2.20±0.10</td>
</tr>
<tr>
<td>7-G</td>
<td>3.40±0.12</td>
<td>3.27±0.12</td>
<td>2.68±0.11</td>
<td>2.53±0.11</td>
<td>2.30±0.10</td>
<td>2.19±0.10</td>
<td>2.08±0.10</td>
</tr>
<tr>
<td>8-G</td>
<td>3.40±0.12</td>
<td>2.51±0.11</td>
<td>1.99±0.09</td>
<td>1.66±0.09</td>
<td>1.48±0.08</td>
<td>1.18±0.08</td>
<td>1.10±0.08</td>
</tr>
</tbody>
</table>

1-G – cardboard box; 2-G – OPP; 3-G – Multibarrier 60 HFP; 4-G – BIALON 50 HFP; 5-G – BIALON 65 HFP; 6-G – ECOLEAN; 7-G – PP; 8-G – BIO NVS

The dynamics of sherbet sample moisture content in MAP (100% CO₂) during the storage time, %

<table>
<thead>
<tr>
<th>Sample</th>
<th>0</th>
<th>2</th>
<th>6</th>
<th>8</th>
<th>12</th>
<th>14</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-AG</td>
<td>3.40±0.12</td>
<td>2.82±0.11</td>
<td>2.46±0.10</td>
<td>2.43±0.10</td>
<td>2.48±0.10</td>
<td>2.41±0.10</td>
<td>2.35±0.10</td>
</tr>
<tr>
<td>3-AG</td>
<td>3.40±0.12</td>
<td>2.98±0.11</td>
<td>3.25±0.12</td>
<td>2.98±0.11</td>
<td>2.63±0.11</td>
<td>2.62±0.11</td>
<td>2.59±0.11</td>
</tr>
<tr>
<td>4-AG</td>
<td>3.40±0.12</td>
<td>2.93±0.11</td>
<td>2.66±0.11</td>
<td>2.56±0.11</td>
<td>2.39±0.10</td>
<td>2.12±0.10</td>
<td>2.03±0.10</td>
</tr>
<tr>
<td>5-AG</td>
<td>3.40±0.12</td>
<td>2.82±0.11</td>
<td>2.44±0.10</td>
<td>2.35±0.10</td>
<td>2.02±0.10</td>
<td>1.77±0.09</td>
<td>1.71±0.09</td>
</tr>
<tr>
<td>6-AG</td>
<td>3.40±0.12</td>
<td>3.14±0.12</td>
<td>2.78±0.11</td>
<td>2.63±0.11</td>
<td>2.71±0.11</td>
<td>2.45±0.10</td>
<td>2.19±0.10</td>
</tr>
<tr>
<td>7-AG</td>
<td>3.40±0.12</td>
<td>3.62±0.12</td>
<td>2.66±0.11</td>
<td>2.70±0.11</td>
<td>2.58±0.11</td>
<td>2.53±0.11</td>
<td>2.49±0.10</td>
</tr>
<tr>
<td>8-AG</td>
<td>3.40±0.12</td>
<td>2.81±0.11</td>
<td>2.10±0.10</td>
<td>1.93±0.09</td>
<td>1.41±0.08</td>
<td>1.31±0.08</td>
<td>1.28±0.08</td>
</tr>
<tr>
<td>9-AG</td>
<td>3.40±0.12</td>
<td>2.87±0.11</td>
<td>2.55±0.11</td>
<td>2.19±0.10</td>
<td>2.03±0.10</td>
<td>1.59±0.09</td>
<td>1.19±0.08</td>
</tr>
<tr>
<td>10-AG</td>
<td>3.40±0.12</td>
<td>3.26±0.12</td>
<td>2.55±0.11</td>
<td>2.21±0.10</td>
<td>1.68±0.09</td>
<td>1.26±0.08</td>
<td>1.23±0.08</td>
</tr>
<tr>
<td>11-AG</td>
<td>3.40±0.12</td>
<td>2.92±0.11</td>
<td>2.52±0.11</td>
<td>2.21±0.10</td>
<td>1.88±0.09</td>
<td>1.47±0.08</td>
<td>1.20±0.08</td>
</tr>
<tr>
<td>12-AG</td>
<td>3.40±0.12</td>
<td>3.04±0.12</td>
<td>2.65±0.11</td>
<td>2.27±0.10</td>
<td>1.95±0.09</td>
<td>1.66±0.09</td>
<td>1.21±0.08</td>
</tr>
<tr>
<td>13-AG</td>
<td>3.40±0.12</td>
<td>3.15±0.12</td>
<td>3.22±0.12</td>
<td>3.16±0.12</td>
<td>3.29±0.12</td>
<td>3.22±0.12</td>
<td>3.21±0.12</td>
</tr>
<tr>
<td>14-AG</td>
<td>3.40±0.12</td>
<td>3.16±0.12</td>
<td>3.20±0.12</td>
<td>3.26±0.12</td>
<td>3.28±0.12</td>
<td>3.27±0.12</td>
<td>3.27±0.12</td>
</tr>
</tbody>
</table>


The dynamics of sherbet sample moisture content in Multibarrier 60 film during the storage, %

<table>
<thead>
<tr>
<th>Sample</th>
<th>0</th>
<th>2</th>
<th>6</th>
<th>8</th>
<th>12</th>
<th>14</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>3.40±0.12</td>
<td>3.17±0.12</td>
<td>2.95±0.11</td>
<td>2.81±0.11</td>
<td>2.55±0.11</td>
<td>2.40±0.10</td>
<td>2.20±0.10</td>
</tr>
<tr>
<td>V</td>
<td>3.40±0.12</td>
<td>3.07±0.12</td>
<td>2.66±0.11</td>
<td>2.59±0.11</td>
<td>2.58±0.11</td>
<td>2.43±0.10</td>
<td>2.29±0.10</td>
</tr>
<tr>
<td>AG-1</td>
<td>3.40±0.12</td>
<td>2.83±0.11</td>
<td>2.62±0.11</td>
<td>2.54±0.11</td>
<td>2.58±0.11</td>
<td>2.51±0.11</td>
<td>2.43±0.10</td>
</tr>
<tr>
<td>AG-2</td>
<td>3.40±0.12</td>
<td>2.83±0.11</td>
<td>2.62±0.11</td>
<td>2.54±0.11</td>
<td>2.58±0.11</td>
<td>2.51±0.11</td>
<td>2.43±0.10</td>
</tr>
<tr>
<td>AG-3</td>
<td>3.40±0.12</td>
<td>2.98±0.11</td>
<td>3.25±0.12</td>
<td>2.98±0.11</td>
<td>2.63±0.11</td>
<td>2.62±0.11</td>
<td>2.59±0.11</td>
</tr>
<tr>
<td>AG-3-A</td>
<td>3.40±0.12</td>
<td>3.30±0.12</td>
<td>3.05±0.12</td>
<td>2.84±0.11</td>
<td>2.78±0.11</td>
<td>2.78±0.11</td>
<td>2.76±0.11</td>
</tr>
</tbody>
</table>

K – cardboard box; G – air ambience; V – vacuum packaging; AG-1 – 30% CO₂+70% N₂; AG-2 – 70% CO₂+30% N₂; AG-3 – 100% CO₂; AG-3-A – 100% CO₂ + oxygen absorber
The dynamics of sherbet moisture content during storage in air ambiance and different packaging materials is represented in Table 3. The initial moisture content of sherbet is 3.40±0.12%, which during storage step by step reduces generates hardening of milk pomade confectionery. The highest moisture content decrease in sherbet samples has been observed in air ambiance and cardboard box (1-G) bulk packaging, as well as in cellulose based biodegradable film BIO NVS (8-G) packaging. The moisture content of samples in BIALON (4-G; 5-G) and BIO NVS (8-G) packaging already after 2 weeks decrease up to 2.51–2.82%, and following up the storage till 16 weeks the moisture decreases below 2.03%. Moisture content of samples packed in PP film pouches during 16 weeks the moisture increase up to 2.08±0.10%. The least decrease of moisture content at the same time is observed in OPP (2-G), Multibarrier 60 HFP; (3-G) and ECOLEAN (6-G) film packed samples, where it reduced till 2.26±0.10%. The dynamics of sherbet moisture content during storage in MAP (100% CO₂) and different packaging materials is represented in Table 4. The influence of biodegradable films is tremendous. Respectively in BIO NVS (8-AG-3), Ceramis®-PLA (9-AG-3), NatureFlex 23NM (10-AG-3); Nativia™ NTSS-30 (11-AG-3) un Nativia™ NZSS-20 (12-AG-3) film packaged samples during storage time 16 weeks the moisture content reduced till 1.19±0.10 to 1.28±0.14%. Accordingly better the moisture of samples remained in conventional BIALON 65 HFP (5-AG-3) packaging – till 1.71±0.11%, BIALON 50 HFP (4-AG-3) – till 2.03±0.05%, OPP (2-AG-3), Multibarrier 60 HFP (3-AG-3), ECOLEAN (6-AG-3) and PP (7-AG-3) respectively till 2.35±0.11%, 2.59±0.16%, 2.19±0.08% and 2.49±0.11%. The samples in met. BOPET/PE (13-AG-3) and Aluthen (14-AG-3) packaging disparate (p<0.05) from all other investigated samples, and the changes in their moisture content during storage are minimal – from initial moisture content 3.40±0.12% till 3.21±0.12% during 16 weeks of storage.

The moisture dynamics of sherbet samples in Multibarrier 60 HFP packaging material with high barrier properties is influenced by various packaging technologies (Table 5). The moisture content of sherbet in cardboard boxes and air ambiance during 16 storage weeks decreased till 1.31±0.08%, while in Multibarrier60 HFP packaging and MAP it ranged within 2.20 to 2.76%. Application of packaging materials with high barrier properties could cut down the moisture migration and increase the shelf life of sherbet (Romeo et al., 2010). The verity alike this is expressed in studies of Londhe, Pal and Raju (2012) concerning Asian candies Peda, which were packed applying different packaging technologies. As a main problem of Peda hardening they consider packaging in air ambiance and cardboard boxes without barriers. Linear regression analysis affirms that close correlation exists between the sherbet hardness and moisture content apart from the packaging technology and material (Fig. 4). The dynamics of moisture content, in its turn, was affected by the packaging material barrier properties, environment composition within the packaging, and presence of oxygen which could be regulated by the oxygen absorbers (active packaging).

![Figure 3](image-url)

**Figure 3. The dynamics of sherbet hardness in Multibarrier 60 HFP material during the storage**

K – cardboard box; G – air ambiance; V – vacuum packaging; AG-1 – 30% CO₂+70% N₂; AG-2 – 70% CO₂+30% N₂; AG-3 – 100% CO₂; AG-3-A – 100% CO₂ + oxygen absorber

The dynamics of sherbet moisture content during storage in air ambiance and different packaging materials is represented in Table 3. The initial moisture content of sherbet is 3.40±0.12%, which during storage step by step reduces generates hardening of milk pomade confectionery. The highest moisture content decrease in sherbet samples has been observed in air ambiance and cardboard box (1-G) bulk packaging, as well as in cellulose based biodegradable film BIO NVS (8-G) packaging. The moisture content of samples in BIALON (4-G; 5-G) and BIO NVS (8-G) packaging already after 2 weeks decrease up to 2.51–2.82%, and following up the storage till 16 weeks the moisture decreases below 2.03%. Moisture content of samples packed in PP film pouches during 16 weeks the moisture increase up to 2.08±0.10%. The least decrease of moisture content at the same time is observed in OPP (2-G), Multibarrier 60 HFP; (3-G) and ECOLEAN (6-G) film packed samples, where it reduced till 2.26±0.10%. The dynamics of sherbert moisture content during storage in MAP (100% CO₂) and different packaging materials is represented in Table 4. The influence of biodegradable films is tremendous. Respectively in BIO NVS (8-AG-3), Ceramis®-PLA (9-AG-3), NatureFlex 23NM (10-AG-3); Nativia™ NTSS-30 (11-AG-3) un Nativia™ NZSS-20 (12-AG-3) film packaged samples during storage time 16 weeks the moisture content reduced till 1.19±0.10 to 1.28±0.14%. Accordingly better the moisture of samples remained in conventional BIALON 65 HFP (5-AG-3) packaging – till 1.71±0.11%, BIALON 50 HFP (4-AG-3) – till 2.03±0.05%, OPP (2-AG-3), Multibarrier 60 HFP (3-AG-3), ECOLEAN (6-AG-3) and PP (7-AG-3) respectively till 2.35±0.11%, 2.59±0.16%, 2.19±0.08% and 2.49±0.11%. The samples in met. BOPET/PE (13-AG-3) and Aluthen (14-AG-3) packaging disparate (p<0.05) from all other investigated samples, and the changes in their moisture content during storage are minimal – from initial moisture content 3.40±0.12% till 3.21±0.12% during 16 weeks of storage.

The moisture dynamics of sherbet samples in Multibarrier 60 HFP packaging material with high barrier properties is influenced by various packaging technologies (Table 5). The moisture content of sherbet

![Figure 4](image-url)

**Figure 4. Correlation of the hardness with moisture content of sherbet**

Research group under the guidance of scientist Romeo (2010) quoted his authorities and justified linkage among moisture content and hardening of product (Romeo et al., 2010). Regression equation obtained by computerized data processing prove on the fact if product moisture content will be reduced by 1%, the hardness will grow by 250 N. The variance analyses performed of scientists Londhe, Pal and Raju (2012) showed on the moisture content disparity among packaging technologies and storage time. Migration of moisture from the product permeating through packaging to environment could be reduced by selecting appropriate packaging material and conditions (Subramaniam, 2000).

The shelf life of sherbet could be extended significantly using packaging materials with high barrier properties: to 10–12 weeks in OPP and Multibarrier60 HFP materials in air ambiance; to 16 weeks in Multibarrier 60 HFP, metallised BOPET/PE and Aluthen materials in MAP (100% CO₂) ambience; as well as to 16 weeks using oxygen absorbers (active packaging) in metallised BOPET/PE and Aluthen materials not exceeding the estimated hardness 300 N. The sherbet hardness in active packaging after 16 weeks in Multibarrier 60 HFP was determined 261.18±8.32 N, in metallised BOPET/PE – 146.01±7.54 N and in Aluthen – 117.61±8.64 N. By the side of packaging in MAP (100% CO₂), in active...
packaging it is for 11.5%, 20% and 35.7% less that can be assessed as positive. Biodegradable polymers with improved barrier properties provide a short-time shelf life of sherbet in air ambience maximum to 4 weeks, in MAP (100% CO₂) – to 6 weeks.

The maximum permissible TPC in sugar confectionery sherbet could be ≤4 log CFU g⁻¹ (SanPin, 2002). After 16 weeks of storage this level has been exceeded only in samples packed in cardboard boxes and OPP film in air ambience. Close to this level, aerobic and facultative anaerobic microorganisms grow in biodegradable Ceramis®-PLA and NatureFlex 23NM packaged sherbet samples. In Multibarrier 60 HFP. met. BOPET/PE and Aluthen packaging, the growth of microorganisms in sherbet samples occurs more slowly. In experimentally analyzed sherbet samples during the storage time, the number of yeast and mould colony forming units has been estimated. The admissible level of both yeasts and moulds is 50 CFU g⁻¹. In any of the tasted samples throughout the storage time, the number of TPC, yeast and mould colony forming units does not exceed the admissible level.

Conclusions

Linear regression analysis prove a close correlation between the sherbet hardness and moisture content in all the studied packaging types, while the dynamics and intensity of moisture content in the product during the storage time are affected by the packaging material barrier properties. The desideratum hardness of sherbet is 300 N, which in cardboard box packaging establishes already after two weeks of storage. Shelf life can be extended significantly in conventional polymer packaging with high barrier properties: to 10-12 weeks in air ambience OPP and Multibarrier 60 HFP packaging: to 16 weeks in MAP 100% CO₂ and Multibarrier 60 HFP. met. BOPET/PE and Aluthen material, exceeding neither experts’ accepted hardness nor permissible total plate count of microorganisms’ level (TPC 6¹⁻>10⁴). The sherbet hardness in active packaging after 16 weeks of storage in Multibarrier 60 HFP is by 11.5% lower than in MAP 100% CO₂, which is valued as positive. Biodegradable polymers with improved barrier properties provide the shelf life of sherbet in air ambience maximum to 4 weeks, in MAP 100% CO₂ maximum to 6 weeks.

References

Abstract
The aim of the research was to study the rheological behavior of candy mass depending on recipes in order to optimize technological regimes for processes of tempering and molding products with specified structural and mechanical properties. The objects of the study were fondant, jelly and whipped semi-finished products made according to the classical recipes and with application of vegetable powders and phytoneutrients. According to the research results, the effect of dosing and dispersion of phytoneutrients and vegetable powders on candy mass viscosity at different temperatures and strain rates allowed to develop recommendations for selecting modes of thermomechanical processing of fondant and jelly masses at the stage of tempering and molding.

It was found that the nature of changes in plastic strength of semi-finished products depends on regimes of structure formation and influence of plant additives on this process. The research showed that powder additives contribute to significant reduction in the duration of structure formation.

The paper also studies the effect of phytoneutrients and vegetable powders on the strength of adhesive contacts between the layers of candy mass in forming multilayered confectionery products. Defined interval of strength values of adhesive contacts allows to prevent delamination and to formulate the main requirements for the conditions and methods for molding candies with a combined body.

The obtained results of complex rheological studies make it possible to implement the evidence-based approach to the management of technological processes of confectionery products manufacture and ensure the achievement of specified technological and consumer characteristics.

Keywords: rheology, adhesion, candy mass, phytoneutrients.

Introduction
Rheological and structure-mechanical characteristics are important indicators of the properties of the candy mass as a semi-finished product coming to further process steps, as well as determine the structural and mechanical characteristics of the finished product. Investigation of adhesion properties allows to evaluate the possibility of using various molding methods or fusion of different physico-chemical and rheological properties of the candy mass for making combined products.

Currently the enrichment of confectionery products with various additives increasing their quality, improving organoleptic and structure-mechanical characteristics of the finished product is becoming increasingly common, as it allows satisfying the human need for biologically active substances. Great contribution to the development of scientific bases of confectionery production using herbal additives were made by famous scientists in this scientific area: Aksenov, Koryachkina, Magomedov, Obolkina, Savenkov, Skobelskaya, etc. In this regard, it is necessary to conduct a thorough rheological study of semi-finished products with the addition of non-traditional raw materials in candy recipes and structure-mechanical characteristics of candies produced from them. The aim of the research is to validate the modes of candy production using the rheological and structural and mechanical properties of semi-finished products.

Materials and Methods
The objects of the study were samples of fondant, jelly and whipped mass and candies made on their basis according to classic recipes, as well as with the addition of vegetable powders (pumpkin, carrot) and medicinal-technical raw materials (nettle leaves, lemon balm, raspberry) of various concentration and dispersion. Herbal additives used in the form of powder, hydrated powders (puree) and water-alcoholic extracts.

The study of rheological properties of semi-finished products and structure-mechanical characteristics of candy bodies were performed on viscosceter HAAKE VT6R plus (Thermo Fisher Scientific, Germany) and texture analyzer Brookfield CT-3 (Brookfield Engineering laboratories, inc., USA) equipped with a wide range of sensors, devices and accessories to meet the challenges associated with the analysis and measurement of texture. Detailed description of methods for analysis of raw materials, semi-finished and finished products is given in (Смолихина, 2013).

Results and Discussion
Studying rheological properties of investigated candy mass has shown that, irrespective of the recipe, they belong to pseudoplastic materials. In a wide range of shear rates 0–100 s⁻¹ the flow of the fondant mass is well approximated by the equation of Herschel-Bulkley, while the jelly and whipped mass satisfies the equation of Ostwald de Waele. In all cases, the rheological curves are well approximated by a linear function over a fairly wide range of shear rates (by a factor of no less than 0.98 approximation), which explains the choice and use of these rheological equations.

The curves of changes in viscosity versus shear rate have a form that is characteristic of structured systems. The viscosity decreases when the shear rate is increasing and especially fast in the range of relatively low shear rates, while the further increase in rate varies viscosity slightly. This rheological property is
explained by the fact that in a stationary environment the arrangement of the particles is characterized by strong randomness, and under the influence of increasing shear forces the orientation of the particles in the flow direction is raising. Increasing the rate leads to decreasing the interaction between particles. Tests were conducted at temperatures typical of molding for this candy mass (Fig. 1).

The viscosity of the whipped mass is five times higher than jelly mass and twice than fondant mass that is due to the presence in the system of a surfactant adsorbed and coagulated to egg protein films forming strong bonds and large amounts of air phase.
Thus, adding dry powder from 2 to 10% (dispersity of 0.14–0.25 mm) into the whipped mass increases the viscosity in 3–10 times, hydrated – in 3.5–11.0 times. The used additives have high adsorption and water-holding capacity, therefore when mixed with the candy mass they swell and form a spatial grid with strong intermolecular bonds. This leads to the “expansion” of the system which causes the reduction in thickness of dispersion environment layers and increase of resistance force, and, accordingly, significant increase in viscosity (Fig. 2). A stronger effect of the hydrated powders, besides the phenomena described above, may be connected with the restoration of powder polymeric structures during its hydration and the dissociation of organic amino acid capable of participating in the jelly forming process which leads to hardening of the mass structure and a high viscosity, respectively.

Adding powdered vegetable semi-finished products of different dispersion into the candy mass significantly changes the rheology of semi-finished products. Decreasing the size of nettle powder particles results in increasing the viscosity of candy mass (Fig. 3). According to the results of studies on rheological properties temperature modes for specific stages of candy production were set (Table 1) (Муратова et al., 2008, 2009).

Plastic strength of candy bodies is a key indicator for characterizing the form retention quality. Changes in strength can show the process of structure formation the rate of which is of great importance for selecting regimes for structure formation process of candy mass (Muratowa, Smolikhina, 2013; Смолихина, Муратова, 2013).

<table>
<thead>
<tr>
<th>Candy mass</th>
<th>Production stages</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whipping</td>
</tr>
<tr>
<td>Fondant</td>
<td>65–75 °C</td>
</tr>
<tr>
<td>Jelly</td>
<td>–</td>
</tr>
<tr>
<td>Whipped</td>
<td>55–65 °C</td>
</tr>
<tr>
<td></td>
<td>New recipe</td>
</tr>
<tr>
<td>Fondant</td>
<td>75–85 °C</td>
</tr>
<tr>
<td>Jelly with the</td>
<td>extract</td>
</tr>
<tr>
<td></td>
<td>powder</td>
</tr>
<tr>
<td>Whipped</td>
<td>–</td>
</tr>
</tbody>
</table>

![Figure 1. Dependence of the candy mass viscosity on shear rate](image1)

![Figure 2. Dependence of the whipped mass viscosity on deformation rate](image2)

![Figure 3. Dependence of the fondant mass viscosity on the shear rate for different dispersion of the nettle powder](image3)

Table 1
The analysis of experimental studies about the effect of functional ingredients on the process of jelly mass formation showed that the use of powder reduces the plastic strength of jelly by 22% (Fig. 4).

Introduction of vegetable powders at the tempering stage of the jelly mass leads to destruction of the jelly monolithic and formation of uneven structure. Polysaccharides of powders that have high sorption properties violate hydrostatic equilibrium while absorbing moisture from the system the result of which becomes an irregular jelly body.

**Figure 4. Changes in the jelly plastic strength during structure formation process**

1 – control, 2 – with the addition of 0.5% pumpkin powder

Introduction of vegetable powders at the tempering stage of the jelly mass leads to destruction of the jelly monolithic and formation of uneven structure. Polysaccharides of powders that have high sorption properties violate hydrostatic equilibrium while absorbing moisture from the system the result of which becomes an irregular jelly body.

When introducing the functional additives with pectin at the syrup preparation stage we can see the maximum dissolving and swelling of the polysaccharide powder. The formed jelly surface is smooth, on the fracture it is glassy with even distribution of insoluble fiber parts (Fig. 5).

**Figure 5. Jelly mass with the addition of vegetable powders 3%**

a – control, b – at the stage of syrup production, c – at the tempering stage

The strength of whipped jellies with the use of powdered semi-finished products, on the contrary, increases due to the combined action of agar molecules and pectin substances presented in vegetable powders in large quantities. Thus, the hydrated powder increases the strength 2.0 fold, the dry powder – 8.0 fold. When using hydrated powder the whipped mass strength increases owing to additional filling of the space frame surrounding the bubbles with swollen fibers of the vegetable powder. Gelation occurs within 40 minutes after casting at a temperature of 20–22 °C, but a large amount of swollen polysaccharides makes the mass aqueous and prone to syneresis.

In samples containing dry powder syneresis is avoided by narrowing the channel, increasing the roughness of the walls and forming local „gates” from the particles not adhered to the bubbles (Зубченко, 2001).

However, the presence of solid particles may have the opposite effect: they may undergo the adsorption of surfactants and the concentration decrease of surfactants in the solution leads to the increase of the surface tension and decrease of the foam dispersion, whereby the syneresis speed can be boosted.

Factors affecting the rate of fondant mass structuring are the ratio of solid and liquid phases, the presence of large crystals, the concentration and dispersion of functional additives, and body temperizing modes. The rate of fondant mass structuring can be judged by the increase in the limit shear stress (Горбатов et al., 1982).

For classical fondant mass at low temperatures (70–75 °C) the limiting shear stress raises dramatically in a short period of time which indicates a high rate of sucrose crystallization. A high degree of supersaturation of the solution leads to intensive crystallization of sucrose not only on the surface but also in internal layers of the body. Structuring process in the mass casting with temperature of 95 °C is slower and the mass cast at temperature of 100 °C reaches normal consistency (critical shear stress of 30–40·10³ N m⁻²) after 3 hours of structure formation process (Зубченко, 1986).

The structure formation process of the fondant mass can be traced according to increase in the strength of the candy body. Figure 6 shows the dependence of the strength of the fondant sample on the depth of the indenter.

**Figure 6. Changes in the consistency of the fondant mass in the process of structuring after casting in**

1 – 15 min, 2 – 35 min, 3 – 60 min.

On the surface of the semi-finished product there is a dense crystalline crust formation the hardness of which increases during the first hour (up to 1600 g), and after two hours of temperizing the thickness reaches 2.5 mm. Inside the formed body there is thick mass
with large crystals of sucrose (the presence of crystals characterizes the presence of peaks within curve) (section 3*). In the crystallization process the adhesion of samples decreases to stainless steel.

In 2.5–3 hours of structure formation process at ambient temperature of 23–25 °C the candy body has a solid crystalline structure with the strength $4 \cdot 10^3$ g (Fig. 7).

The study on adhesive properties of the candy mass helps to evaluate the possibility of using various molding techniques for the manufacture of candy bodies.

The violation of production modes, moisture migration between the layers and syneresis during the storage leads to weakening of the adhesive interactions and changing in structural and mechanical characteristics of the products.

While forming the adhesive bonding through the combination of jelly and whipped semi-finished products in the combined body, highly viscous masses come into contact. In this case, for describing the process of contact formation rheological characteristics of the adhesive and the terms of the contact become important depending on the method of molding semi-finished products.

The molding of combined jelly-whipped candies can be produced with methods of co-extrusion, smearing followed by cutting, and casting.

The practice of molding by co-extrusion method showed that for each production it is necessary to make corrections of technological modes taking into account the constructive features of the molding equipment, or using them for combining confectionery masses close by rheological characteristics and having thixotropic properties (Оболкина et al., 2008).

The formation of candies by casting and smearing minimizes mechanical effect on the formable candy mass which does not destroy their structure and reduces the residual stress at the phase boundary. The smearing method allows to make multilayer products, but in the process of cutting there may be possible violations of structures, displacement of the layers due to their deformation (Fig. 8).

The formation of jelly-whipped candies was performed by the smearing method followed by cutting and casting. The ability to control rheological properties of the jelly mass by changing the temperature at the molding stage is a determining factor in the choice of jelly layer as the top. When the temperature is increased, the viscosity of the jelly mass decreases non-linearly with average increase of temperature by 1 °C per 0.01 Pa·s (Леонов, Муратова, 2011). At low viscosity the liquid adhesive wets the surface of the substrate providing a flawless full contact with the whipped mass with the maximum filling of micropores on the surface, but there is a formation of adhesive interaction and hardening of adhesive contact. However, even in this case, the adhesive strength of the contact is insufficient to prevent full separation of the structure by mechanical action on the semi-finished product during cutting the layer obtained by smearing or during removing candy bodies made by the method of casting to the starch forms (Fig. 9).

When adding vegetable powders in the whipped layer in the amount of less than 2%, the adhesive strength between the layers of the body does not differ significantly from indicators obtained for the combined bodies without additives. Introducing powders into the whipped layer in the amount more than 10 wt. % leads to excessive development of microrelief which adversely affects the achievement of maximum contact area: a large number of connections reduces the mobility of macromolecules in the boundary layer, increases internal pressure, changes the structure of the surface layer, which results in defective areas that serve...
as centers where the destruction of adhesive compounds begins (Зимон, Евтушенко, 2008; Смолихина, Муратова, 2012). The maximum adhesion between the layers of candy mass is attained by adding vegetable powders in the whipped layer in the amount of 5–10 wt.%, in the jelly layer – less than 3%, while the strength of adhesion contacts increases by more than 30% compared to control samples (Fig. 10 and 11).

Based on the above, it can be concluded that adding the carrot powder into the whipped mass allows to improve the contact area of the adhesive-substrate and to increase the adhesive connection strength due to the formation of a rough surface of the whipped mass and maximum filling of microdefects in the jelly mass. Moreover, the persistence of structural and mechanical properties of the whipped mass in the result of enhancing foam frame by rough fibers of the carrot powder provides the structuring capacity of the whipped layer and allows the operation of casting the jelly mass with density of 1350 kg/m³ at 45–60 minutes after the formation. Vegetable powders having high water-binding capacity absorb moisture from the candy mass surface to improve their adhesion to the combinable layer. Adding powders into the jelly mass reduces jelly strength and allows to change the adhesive tense on its surface which has a positive impact on the quality of the adhesive contact with the whipped mass (Смолихина, Муратова, 2013).

The studies have shown that during tests of control samples there is usually a mixed adhesive breaking, and that during tests of samples with the addition of vegetable powders the breaking is predominantly cohesive.

Based on the influence of phytonutrients dependencies on structural and mechanical characteristics of the fondant and whipped masses, the recommendations were made for the conditions and methods of molding (Table 2).

### Table 2

<table>
<thead>
<tr>
<th>Molding temperature, °C</th>
<th>Candy mass</th>
<th>Control</th>
<th>New recipe</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fondant mass</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;70</td>
<td>Casting followed by prolonged structure formation process</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40–70</td>
<td>Casting, smearing, pressing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25–40</td>
<td>Smearing, pressing</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Whipped mass</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;60</td>
<td>Casting</td>
<td></td>
<td></td>
</tr>
<tr>
<td>45–60</td>
<td>Casting, smearing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30–45</td>
<td>Smearing, pressing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25–30</td>
<td>Smearing, pressing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>Not recommended</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

When the molding temperature is above 70 °C, the fondant structuring occurs after casting the bodies, thus the shear rate in the molding can be set arbitrarily. Temporizing of the bodies proceeds to a temperature of 20–22 °C for 35–40 minutes. Structuring process is characterized by the formation of crystallization centers with the addition of new molecules to the grid and proceeds spontaneously as it is accompanied by decreasing free energy of the system. When using the fondant mass as fillings at the temperature range of 40–70 °C, their supply to product bodies may be performed at any shear rate. While molding the fondant mass at temperatures below 40 °C in order to avoid destroying the structure of the mass, the shear rate should be less than the lowest critical rate, i.e. 25 s⁻¹.

Structuring occurs after casting of the whipped mass at high temperatures, so the shear rate during the formation process can be set arbitrarily. At temperatures below 60 °C the process of jelly formation begins, therefore the impact on the structure should be minimal. This requirement corresponds to the smearing method. For the temperature range of 30–45 °C the whipped mass made according to traditional recipes should be formed by smearing.
while for the whipped mass with the addition of vegetable powders the pressing method can be used because the moulded products retain their shape well in the form of a slice. If the temperature is 25–30 °C the whipped mass with the addition of vegetable powders can be molded by settling. Thus molded product retains their shape well as the process of structure formation by the time of molding has largely been completed. To prevent the destruction of the mass structure during the molding the shear rate should be below the lowest critical rate, i.e. 30 s⁻¹.

Conclusions
Research results testify to operational parameters for candy mass forming which can be recommended for practical use in the candies production according to traditional recipes and adding powders and herbal extracts. It is found that rheological behavior in the range of shift rates studied is similar to mixture composition which contains powders of medicative, technical and vegetable raw materials characterized by similar parameters of water-retaining and adsorption capacities. Thus, the results gained could be recommended not only for candies production with above mentioned additives but also for using different herbal additives with the same physical and chemical properties.

Acknowledgment
The authors thank the Director for quality of JSC „Confectionery company „TAKF” N.V. Donskih for assistance in conducting laboratory tests and industrial experiments, the Candidate of Engineering D.V. Leonov for constructive cooperation when carrying out rheological studies of jelly and fondant semi-finished products and the Candidate of Philology E.Yu. Voyakina for translation of this article.

References
DETERMINATION OF ACRYLAMIDE LEVELS IN SELECTED FOODS IN LATVIA AND ASSESSMENT OF THE POPULATION INTAKE

Iveta Pugajeva1,2, Laura Zumbure1, Aija Melngaile1, Vadims Bartkevics1,2

1 Institute of Food Safety, Animal Health and Environment BIOR, Lejupes street 3, Riga, Latvia, e-mail: iveta.pugajeva@bior.gov.lv
2 University of Latvia, Faculty of Chemistry, Kr. Valdemara street 48, Riga, Latvia

Abstract

The aim of this study was to investigate acrylamide levels in foods obtained from the Latvian market. Eight sample groups including traditional Latvian food products (rye bread, rye bread enriched with seeds, dried fruits or vegetables, fine rye bread (sweet-and-sour bread), wheat bread, potato chips, coffee, pastry products, dried bread products and gingerbread) were analyzed for their acrylamide content. A total of 435 samples were analyzed for acrylamide concentration using the ultra high performance liquid chromatography with tandem mass spectrometric detector and QuEChERS sample preparation methodology. An appropriate average recovery (106%), within-laboratory repeatability (3–10%) and limit of quantification (10 µg kg ) was specific to the applied UPLC-MS/MS methodology. Results revealed that the acrylamide content of processed foods shows a great variation between different food groups as well as between brands and within brands. Highest concentration of acrylamide was revealed in potato chips and chicory coffee with average concentration 564 µg kg and 2790 µg kg respectively. No single bread sample has exceeded the European Commission Recommendation 2013/647/EU although the addition of seeds, fruits or vegetables clearly had an increasing effect on acrylamide concentration in bread. The dietary exposure assessment was performed, using analytical results on acrylamide levels in certain food groups and the relevant food consumption data. The calculated data indicates high dietary intake of acrylamide by certain consumers that may potentially cause adverse health effects. Therefore, adequate efforts should be made to diminish acrylamide levels in processed foods in order to reduce the dietary risk to the human health for Latvian population.

Keywords: acrylamide, Latvian food, population intake.

Introduction

Acrylamide, a thermal process-induced contaminant in food, has attracted worldwide researchers to study the mechanism of its formation in foods, the risks associated for consumers and possible strategies to lower acrylamide levels in foodstuffs (Lui et al., 2013). The International Agency for Research on Cancer, taking into consideration an option of industrial exposure to this compound and its intake from drinking water and tobacco smoke, classified acrylamide already in 1994 as a compound „probably carcinogenic for humans“ (Group 2A) (IARC, 1994). Experimental studies in animals have shown genotoxic and carcinogenic effects of acrylamide. The genotoxicity of acrylamide could be partly mediated by its main metabolite, glycidamide (Gamboa da Costa et al., 2003; Baum et al., 2005) which is mutagenic (Blasiak et al., 2004; Ghanayem et al., 2005).

Since April 2002, when the Swedish National Food Administration published for the first data set about high content of acrylamide in food, several studies in different countries were performed dedicated to the assessment of acrylamide content in foods. The presence of acrylamide in different foodstuffs and its toxic impact, stirred up interest on a global scale in a possible risk to human health. Consequently, many countries have started to estimate acrylamide dietary intake (Mojska et al., 2012).

Currently it is well known that acrylamide is mainly formed as a result of reaction between amino acids and reducing sugars (particularly glucose and fructose) as a part of Maillard reaction (Mottram et al., 2002; Stadler, Scholz, 2004). Studies also have shown that acrylamide formation is affected by several processing parameters, such as heating temperatures, duration of heating, reducing sugar content, addition of components that bind water, and surface to volume ration (Boon et al., 2005).

The main source of acrylamide in the diet are primarily products made of potato such as French fries and potato crisps, and also cereal products such as bread, breakfast cereals, cookies and biscuits. Acrylamide is also formed during the coffee roasting process (Mojska et al., 2012).

Due to multiple sources of dietary exposure to acrylamide, a consultation was held in June 2002 by the Food and Agriculture Organization/World Health Organization (FAO/WHO) to discuss possible health risks due to acrylamide (WHO, 2002). Based on the expert committee evaluation, 1 µg kg body weight (bw) day is considered as an average exposure to acrylamide while 4 µg kg bw day is considered as high exposure to acrylamide (JECFA, 2006). The mean dietary exposure range to acrylamide is 0.2–1.0 µg kg bw day for the general adult population while 95th percentile range is 0.6–1.8 µg kg bw day (JECFA, 2011).

Currently, acrylamide is monitored in the European Union according to the European Commission Recommendation (2010/307/EU). Indicative acrylamide values based on the EFSA (European Food Safety Authority) monitoring data from 2011 to 2012 were established within the European Commission recommendation released on November 8, 2013 (“On investigations into the levels of acrylamide in food”). The indicative values are not safety thresholds, but, if exceeded, subsequent investigation of the reasons should be conducted.

To sum up significant variation of acrylamide content within the Latvian food products was obtained mainly as a result of the variable critical process parameters (critical temperature threshold, processing time, etc.) which are characteristic for each type of food.
The content of acrylamide in traditional Latvian origin products (rye bread and rye bread with addition of seeds and fruit) is in line with the EU recommendation 2010/307/EU. The data presented here for other Latvian foods are in the same range as published in other countries. The aim of this study was to investigate acrylamide levels in foods obtained from the Latvian market and to assess the average dietary acrylamide exposure of the Latvian population.

Materials and Methods

Food sampling

Given that acrylamide is formed in heat-treated potato and cereal products such as bread, breakfast cereals, cookies and biscuits and during the coffee roasting process, the food products produced in Latvia were selected for testing purposes that might be a prospective source of dietary acrylamide intake for the Latvian population.

Eight product groups were selected for the study:
1. rye bread, rye bread enriched with seeds, dried fruits or vegetables;
2. sweet- and-sour bread;
3. wheat bread;
4. potato chips;
5. coffee, including instant;
6. confectionery (cookies, crackers);
7. gingerbread;
8. bread products (bagels, crackers, etc.).

A total of 435 samples obtained from all Latvian regions were analyzed for the acrylamide content.

Chemicals

Acrylamide (99%) and acrylamide-d₃ standards were obtained from Sigma (St. Louis, MO, USA). Stock and working standard solutions of acrylamide and acrylamide-d₃ were prepared in acetonitrile. Methanol, hexane and acetonitrile were a gradient grade for HPLC and formic acid (98%) from Sigma (St. Louis, MO, USA). Acrylamide (99%) and acrylamide-d₃ standards were prepared in acetonitrile. Methanol (6%) solution in acetonitrile acidified with 0.1% formic acid was used as a mobile phase (flow rate 0.25 mL min⁻¹, column temperature 40 °C and injection volume 10 µL). The conditions for the detection by MS/MS were as follows: 30 psi curtain gas (CUR), 5500 V ion spray voltage, 400 °C temperature, 40 psi ion source gas (GS1), 50 psi ion source gas (GS2), 60 V declustering potential (DP).

Acrylamide was quantified in food samples using the product ion m/z 55 (precursor ion m/z 72) for the analyte and m/z 58 (precursor ion m/z 75) for the internal standard (acrylamide-d₃). The quantification was based on the peak area of analyte compared to that of the deuterated internal standard.

The limit of quantification for acrylamide was 10 µg kg⁻¹.

Quality control

The LC-MS/MS method for determination of acrylamide in food was validated. The following parameters were defined and tested: limit of quantification (LOQ), within-day precision, between-day precision and recovery. The laboratory has participated in the inter-laboratory proficiency testing organized in 2011 by FAPAS analyzing a biscuit test material and has received a Z-score -0.3. Furthermore, the analytical quality control sample was also used in each series of analysis to determine recovery of acrylamide during the sample preparation procedure.

Food consumption data collection

The comprehensive National Food Consumption database was developed in 2007-2009 and is updated from 2012-2013 to facilitate:
- risk assessment in relation to chemical and biological hazards in food,
- estimation of nutrient intakes by target population,
- development of scientifically based food policy documents.

During the first stage of the fieldwork in total 2000 individuals aged 19 to 64 years old and living in private households in Latvia were interviewed. Three age groups (19–35, 36–50, 51–64) were created. Information on food intake for each age group was collected using two non-consecutive 24 h recalls in combination with food frequency questionnaire. Additional data on sociodemographic and lifestyle characteristics were obtained in face-to-face interviewing process. Nutritional data were processed with the help of software for the storage and analysis of food consumption and composition data that was developed in frame of this project.
Design and the methodology of the food consumption survey was based on „European Food Consumption Survey Method (EFCONS)“ recommendations (EFCONS, 2002), developed within the framework of the EU Programme on Health Monitoring to provide the common method for monitoring of food consumption in Europe in a comparable way.

Strictly standardized procedures were used in order to harmonise research methodology (including sample size, data analysis and data presentation) and to obtain data comparable with the data from other EU countries. For the quantification of portion sizes the food picture book was used. For presentation of the data, parameters of interest were mean, median, quartiles, P5 and P95.

The exposure assessment on the base of National consumption data has been conducted concerning chemical contamination risk and related nutrition risk to develop scientifically based conclusions and to ensure relevant risk management and communication activities.

At present very limited information about the content of acrylamide in Latvian origin food is available, therefore, the main objective of our study was to determine the content of this compound in the Latvian foods and to assess the average dietary acrylamide exposure of the Latvian population.

Results and Discussion

The acrylamide content in 435 samples of food manufactured in Latvia ranged from 8 to 2790 μg kg⁻¹ of foodstuff. High standard deviation indicates significant variation of results obtained for individual product groups.

All food products samples were divided into three groups based on the mean value of acrylamide (<100 μg kg⁻¹, from 100 to 200 μg kg⁻¹ and from 230 to 900 μg kg⁻¹). The highest mean acrylamide content falling into the range from 200 to 900 μg kg⁻¹ was obtained for the following product groups: crisps, grain biscuits, gingerbread and coffee. The highest variations were observed in the group of crisps – from 42 to 1570 μg kg⁻¹. Relatively higher acrylamide content was determined in crisps with added flavour combinations and crisps produced, using potato varieties with high sugar content. The studies on mechanisms of the acrylamide formation (Taeymans et al, 2004) show that the increased presence of reducing sugars and fats significantly increases the acrylamide formation.

Our results were similar to those obtained by Eerola et al. (2007) in Finland (539 μg kg⁻¹), Murkovic (2004) in Austria (627 μg kg⁻¹) and EFSA (2011) (635 μg kg⁻¹). Slightly higher mean content of acrylamide in crisps was observed by Mojska et al. (2010) in Poland (904 μg kg⁻¹) and Sirot et al. (2012) in France (954 μg kg⁻¹). In all quoted studies the high acrylamide content variation within product groups was observed. Mean acrylamide value in the range from 100 to 200 μg kg⁻¹ was obtained for five product groups: bagels, biscuits, chocolate biscuits, savoury biscuits and puff pastry.

Table 1

<table>
<thead>
<tr>
<th>Product description</th>
<th>N</th>
<th>Mean</th>
<th>S.D.</th>
<th>Median</th>
<th>Range, μg kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bread</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bread wheat</td>
<td>48</td>
<td>14</td>
<td>6</td>
<td>12</td>
<td>&lt;10–36</td>
</tr>
<tr>
<td>Bread wheat with additives</td>
<td>11</td>
<td>29</td>
<td>16</td>
<td>31</td>
<td>10–54</td>
</tr>
<tr>
<td>Bread rye</td>
<td>77</td>
<td>48</td>
<td>16</td>
<td>47</td>
<td>14–87</td>
</tr>
<tr>
<td>Bread sweet sour</td>
<td>47</td>
<td>28</td>
<td>20</td>
<td>25</td>
<td>&lt;10–133</td>
</tr>
<tr>
<td>Bread rye with additives</td>
<td>33</td>
<td>54</td>
<td>30</td>
<td>52</td>
<td>14–152</td>
</tr>
<tr>
<td>Rusk</td>
<td>16</td>
<td>65</td>
<td>27</td>
<td>57</td>
<td>27–93</td>
</tr>
<tr>
<td>Toast, rye bread</td>
<td>12</td>
<td>40</td>
<td>17</td>
<td>37</td>
<td>20–79</td>
</tr>
<tr>
<td>Snacks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crisps</td>
<td>55</td>
<td>564</td>
<td>517</td>
<td>303</td>
<td>42–1570</td>
</tr>
<tr>
<td>Bagels</td>
<td>22</td>
<td>162</td>
<td>128</td>
<td>119</td>
<td>39–588</td>
</tr>
<tr>
<td>Coffee</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roasted</td>
<td>13</td>
<td>450</td>
<td>120</td>
<td>450</td>
<td>300–600</td>
</tr>
<tr>
<td>Soluble</td>
<td>4</td>
<td>900</td>
<td>110</td>
<td>920</td>
<td>790–980</td>
</tr>
<tr>
<td>Chicory</td>
<td>1</td>
<td>2790</td>
<td></td>
<td></td>
<td>2790</td>
</tr>
<tr>
<td>Pastry</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biscuit</td>
<td>53</td>
<td>187</td>
<td>118</td>
<td>91</td>
<td>&lt;10–1060</td>
</tr>
<tr>
<td>Biscuit, nuts</td>
<td>7</td>
<td>97</td>
<td>90</td>
<td>68</td>
<td>&lt;10–279</td>
</tr>
<tr>
<td>Biscuits, grain</td>
<td>7</td>
<td>400</td>
<td>165</td>
<td>393</td>
<td>147–606</td>
</tr>
<tr>
<td>Biscuit, chocolate</td>
<td>6</td>
<td>141</td>
<td>122</td>
<td>115</td>
<td>23–341</td>
</tr>
<tr>
<td>Biscuits, savoury</td>
<td>8</td>
<td>125</td>
<td>123</td>
<td>66</td>
<td>&lt;10–322</td>
</tr>
<tr>
<td>Butter</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>biscuit, toffee</td>
<td>4</td>
<td>28</td>
<td>9</td>
<td>26</td>
<td>19–41</td>
</tr>
<tr>
<td>Gingerbread</td>
<td>2</td>
<td>238</td>
<td>59</td>
<td>238</td>
<td>196–280</td>
</tr>
<tr>
<td>Puff pastry</td>
<td>9</td>
<td>102</td>
<td>62</td>
<td>82</td>
<td>20–200</td>
</tr>
</tbody>
</table>

This group also showed rather high variation of the acrylamide content. Similar values have been reported by other authors (Murkovic, 2004; Rufian-Henares, 2007) with revealed acrylamide levels up to 2085 μg kg⁻¹. Biscuits and bagels produced with addition of different components, e.g. sesame, bran, poppies, and onions, contained higher levels of acrylamide.

The lowest acrylamide content was found in bread, bread products, biscuits with nuts and toffee. It should be noted that the acrylamide content in bread with addition of seed and dried fruit was significantly higher in comparison with samples of bread without any additives and this conclusion complies with the study of Taeymans, Wood et al, 2004 about the acrylamide content in lipid rich foods.
## Table 2

Estimation of the acrylamide exposure for adults aged 19–35 years

<table>
<thead>
<tr>
<th>Food products</th>
<th>Consumption, g per person per day</th>
<th>Acrylamide content, µg g(^{-1})</th>
<th>Exposure assessment, µg per person per day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Min</td>
<td>Max</td>
</tr>
<tr>
<td>Rye bread</td>
<td>54.51</td>
<td>0.16</td>
<td>900.00</td>
</tr>
<tr>
<td>Rye bread with dried fruits</td>
<td>54.51</td>
<td>0.16</td>
<td>900.00</td>
</tr>
<tr>
<td>Sweet and sour bread</td>
<td>27.14</td>
<td>0.03</td>
<td>375.00</td>
</tr>
<tr>
<td>Wheat bread</td>
<td>52.57</td>
<td>0.05</td>
<td>1500.00</td>
</tr>
<tr>
<td>Cookies</td>
<td>11.29</td>
<td>0.03</td>
<td>213.70</td>
</tr>
<tr>
<td>Potatoes chips</td>
<td>9.90</td>
<td>0.01</td>
<td>136.88</td>
</tr>
<tr>
<td>Coffee</td>
<td>6.61</td>
<td>0.00</td>
<td>39.42</td>
</tr>
</tbody>
</table>

## Table 3

Estimation of the acrylamide exposure for adults aged 36–50 years

<table>
<thead>
<tr>
<th>Food products</th>
<th>Consumption, g per person per day</th>
<th>Acrylamide content, µg g(^{-1})</th>
<th>Exposure assessment, µg per person per day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Min</td>
<td>Max</td>
</tr>
<tr>
<td>Rye bread</td>
<td>57.37</td>
<td>0.14</td>
<td>1600.00</td>
</tr>
<tr>
<td>Rye bread with dried fruits</td>
<td>57.37</td>
<td>0.14</td>
<td>1600.00</td>
</tr>
<tr>
<td>Sweet and sour bread</td>
<td>34.93</td>
<td>0.07</td>
<td>625.00</td>
</tr>
<tr>
<td>Wheat bread</td>
<td>58.31</td>
<td>0.07</td>
<td>750.00</td>
</tr>
<tr>
<td>Cookies</td>
<td>11.56</td>
<td>0.05</td>
<td>300.00</td>
</tr>
<tr>
<td>Potatoes chips</td>
<td>2.79</td>
<td>0.05</td>
<td>78.36</td>
</tr>
<tr>
<td>Coffee</td>
<td>9.06</td>
<td>0.01</td>
<td>231.73</td>
</tr>
</tbody>
</table>

## Table 4

Estimation of the acrylamide exposure for adults aged 51–64 years

<table>
<thead>
<tr>
<th>Food products</th>
<th>Consumption, g per person per day</th>
<th>Acrylamide content, µg g(^{-1})</th>
<th>Exposure assessment, µg per person per day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Min</td>
<td>Max</td>
</tr>
<tr>
<td>Rye bread</td>
<td>86.93</td>
<td>0.16</td>
<td>810.00</td>
</tr>
<tr>
<td>Rye bread with dried fruits</td>
<td>86.93</td>
<td>0.16</td>
<td>810.00</td>
</tr>
<tr>
<td>Sweet and sour bread</td>
<td>41.48</td>
<td>0.08</td>
<td>750.00</td>
</tr>
<tr>
<td>Wheat bread</td>
<td>51.41</td>
<td>0.05</td>
<td>470.14</td>
</tr>
<tr>
<td>Cookies</td>
<td>11.84</td>
<td>0.05</td>
<td>225.01</td>
</tr>
<tr>
<td>Potatoes chips</td>
<td>2.11</td>
<td>0.03</td>
<td>58.77</td>
</tr>
<tr>
<td>Coffee</td>
<td>8.49</td>
<td>0.01</td>
<td>70.52</td>
</tr>
</tbody>
</table>

Assessment of Latvian population exposure to acrylamide intake from food products

The mean daily consumption of seven product groups in which acrylamide intake was determined analytically and estimated exposure to dietary acrylamide intake for three Latvian individual age groups (adults 19–35 years, 36–50 years and 51–64 years) is presented in Table 2, 3 and 4. Based on
the results of the actual daily consumption, out of all in the highest quantity. In the oldest population group (51–64 years) the consumption of bread was 179 g per person per day. The highest consumption of potato chips is observed for adults group 19–35 years (9.9 g per person per day). Mean consumption of coffee was equal for all groups of population with the high variability between individuals. The mean daily dietary acrylamide intake ranged from 17 μg per day in the adults group aged 19–35 years and 51–64 years to 18.7 μg per day in the adults group aged 36–50 years. The acrylamide intake from food originated mainly in three product groups. In the Latvian population bread supplied 31% of the total dietary acrylamide intake in the group of adults aged 19–35 years, 33% in the group of adults aged 36–50 years and even up to 46% in the group of adults 51–64 years. Potato chips on average supplied 16% of acrylamide in the adult population (19–64 years) with significant difference between age groups (from 32% in the group of adults aged 19–35 years to only 7% in the group of aged 51–64 years). The significant source of dietary acrylamide in the Latvian population was also coffee that supplied 40% of total dietary acrylamide intake in the adult population.

The calculated average dietary acrylamide exposure for the Latvian population aged 19–64 years is 0.26 μg kg⁻¹ of body weight per day and this estimation is in good agreement with the WHO data showing that the average dietary acrylamide exposure for the general population ranges between 0.3 and 0.8 μg kg⁻¹ of body weight per day (WHO, 2002). However it should be taken into account that not all foodstuffs that might contain acrylamide were regarded in our study including home-made products that might represent a major source of dietary acrylamide intake. Therefore the estimated acrylamide intake is probably lower than actual and further research is necessary. Besides it is disturbing to note that for the high consumer group the estimated exposure to dietary acrylamide intake exceeds even ten fold the estimate mean exposure for the total population (up to 3 μg kg⁻¹ of body weight per day). This level coincide with the tolerable daily intake (TDI) for cancer estimated in other study (Tardiff et al., 2012) and therefore high dietary intake of acrylamide by certain consumers may potentially cause adverse health effects and certain activities should be made to diminish acrylamide levels in processed foods with elevated content of this compound in order to reduce the dietary risk to the human health for Latvian population.

Conclusions
A survey of the food products produced in Latvia was performed for determining the acrylamide contents in these products. The obtained data on content of acrylamide in foodstuffs was used to evaluate the dietary exposure estimates of the Latvian population. The highest level of acrylamide was detected in potato crisps and chicory coffee with average concentration 546 μg kg⁻¹ and 2790 μg kg⁻¹ respectively. In general, analysed product groups, bread was consumed crisps, biscuits and coffee were among the food products with the high level of acrylamide. Significant differences were observed in the acrylamide contents between different brands and within brands of the snacks and biscuits. The addition of seeds, fruits or vegetables in bread had an increasing effect on acrylamide content.

References
ACRYLAMIDE REDUCTION OPTIONS IN RYE BREAD
Daiga Kunkulberga¹, Ilga Gedrovica², Vija Ozolina³, Inga Ciprovica¹, Vita Sterna³

¹ Faculty of Food Technology, Latvia University of Agriculture, Liela street 2, Jelgava, Latvia, e-mail: daiga.kunkulberga@llu.lv
² Forest Faculty, Latvia University of Agriculture, Akademijas street 11, Jelgava, Latvia
³ State Stende Cereals Breeding Institute, Dizstende, Libagi parish, Talsi, Latvia

Abstract
Acrylamide is a food contaminant that can be formed in foods when heated above 120 °C, if carbohydrates, especially reducing sugars, and asparagine are present. Bread is among the products that can contain high levels of acrylamide. The aim of this study is to evaluate acrylamide content in bread samples from different varieties of rye grains from different soils and to analyse acrylamide content reduction possibilities. Within the framework of the research, soil sulphur sufficiency in experimental fields of rye were analysed, and the content of amino acids was determined in rye grains, dough and bread by using standard methods. The samples were obtained from rye grain, flour and bread, then the impact of enzyme asparaginase, rosemary extract, and citric acid on the acrylamide content in the bread were analysed. The rye bread baking tests were carried out in an industrial bakery. The results of the soil analysis indicated a deficiency of sulphur containing compounds. The usage of enzyme asparaginase in rye bread production caused no significant reduction (p>0.05) of acrylamide content in rye bread. Analysis of the economic aspects of asparaginase utilization concluded that its utilization in production is not cost effective. The addition of rosemary extract to rye dough did not reduce the acrylamide content in bread. The addition of citric acid to rye dough reduced the content of acrylamide in rye bread by 66%, but had a negative effect on taste. Therefore, it is necessary to find the optimum quantity of citric acid that can be added to bread without changing the sensory properties of acidity and flavour.

Keywords: rye bread, acrylamide, asparaginase, citric acid, rosemary extract.

Introduction
Swedish scientists found acrylamide in food in 2002 (Yuan, 2011; Claus et al., 2008a). At present, a lot of research has been carried out in the world to understand its formation mechanism and to reduce the content of acrylamide in foodstuffs. Investigations made by Maastricht university (Hogervorst et al., 2007), indicate close correlation between acrylamide quantity in food and cause of separate dangerous disease. The International Agency for Research on Cancer is classified acrylamide as probably carcinogenic (2A group) to humans (IARC, 1994), however indistinctness still exist in that matter, because several investigations deny it (Laroque et al., 2008). Acrylamide is substance, which may be form in starch containing products processed in high temperature (above 120 °C) – by fritting, frying, toasting. The most concentration of acrylamide is in bread’s crust (Brathen, Knutsen, 2005). Several scientists concluded that acrylamide forms during Maillard reaction (Zyzak et al., 2003; Stadler et al., 2002; Becalski et al., 2003). Investigations showed that acrylamide does not form in boiled starch containing products. It is possible decomposing of acrylamide at protractedly frying at high (200 °C) temperature (Ryderberg at al., 2003, Ahrne at al., 2007). Factors what promote formation of acrylamide in foodstuffs are different, and strengthening each other. The most important of these is presence of amino acid asparaginase, reducing sugar fructose as well as temperature, treatment time, water activity. Asparagine (Asn) is neutral amino acid and results of scientific investigations show that it is one of the main causes of acrylamide in bread and bakery products (Zhang et al., 2009).

The ratio of N : C in asparagin molecule is 2 : 4 which provide reserve and transportation of nitrogen in plants (Lea, 2007). Asparagine accumulates in plants under stress conditions. The growth of asparagine by 50% in wheat is observed under sulphur deficiency conditions. Cereals have been little studied in this area but existing investigations show that genetic as well as environmental factors dramatically affect asparagine in cereals (Zhang et al., 2009, Halford et al., 2007). Latvia no data about asparagine content in grain products, up to now in amino acids analysis was determined acidic amino acid - aspartic acid (Asp).

Swedish researchers found that the addition of asparagine dough stated notable acrylamide growth in bread from 80 to 6000 ppm, 99% of this amount was in crust (Yaylayan et al., 2003; Mottram et al., 2002). European Food Safety Authority (EFSA) Scientific Panel on 19 April 2005 adopted statement on acrylamide in food where confirmed the need for the risk assessment. The EU has not imposed restrictions on the acrylamide concentration in food but has developed recommendations for its reduction, as well as EFSA incorporated data from manufacturers about its content in food. EFSA acrylamide indicative figures mentioned that fresh bread should not exceed 150 μg kg⁻¹, crisp-bread – 500 μg kg⁻¹ of acrylamide (EFSA, 2009).

The Scientific literature (Claus et al., 2008a) data on acrylamide content in bread and bakery products has wide range due not only to different bread recipes and dough making technology but also to the kind of oven used and the kind of baking process. Agromonical measures are recommended carry out in order to reduce acrylamide content in bread as well as make changes in the recipe and technology. Scientists recommended controlling sulphur level in the soil during cereals breeding as agronomical measure. This is reflected in the study where sulphate deficiency in
cereals during breeding period called dramatic increases in the concentration of asparagine in grains and unexpectedly high acrylamide content in bread (Muttucumaru et al., 2006; Claus et al., 2008b).

German scientists investigating asparagine in rye have found that it is 319–791 mg kg⁻¹ (Springer et al., 2003). There were no investigations in Latvia about asparagine content in rye and bread but there are data about aspartic acid content in grain samples of five rye varieties as 0.55–0.70 g 100 g⁻¹ (Straumite, 2006, Ozolina, 2012).

Following the guidelines of CIAA, would be useful to develop cooperation between farmers, manufacturer and scientists on sulphur containing fertilizers utilization on cereals cultivation, as well as on new cereals varieties containing as low as possible amount of asparagines (CIAA, 2009).

The aim of study is to evaluate acrylamide content in bread samples from different varieties of rye grains and to analyse acrylamide content reduction possibilities. Within the framework of the research, soil sulphur sufficiency in experimental fields of rye was analysed, and the content of amino acids was determined in rye grains, dough and bread. The samples were obtained from rye bread baking tests, then the impact of enzyme asparaginase, rosemary extract, and citric acid on the acrylamide content in the bread was analysed. The rye bread baking tests were carried out in an industrial bakery. The content of amino acids in dough and bread was determined, and aspartic acid relevance to acrylamide formation was evaluated.

Materials and Methods

The Influence of Meteorological Conditions
Winter rye was sown in late September in weather conditions favourable to winter crops.

Snow cover formed in the last ten days of October yet did not cause significant damage and winter rye growth continued until the second ten days of November. At the end of the active growth period, winter crops had begun their tillering phase. In total, the winter months were colder and richer in precipitation compared with long-term mean data.

As the ground under the snow was not frozen, it delayed the plants overheating under a thick snow blanket ensuring their survival. In the spring of 2013 winter receded slowly, in the first ten days of April the average temperature was negative, the winter crops recrudesced in the second ten day period of April which is about a week later than usual. April was cool, the warm weather arrived in the first ten days of May, the second and third ten-day periods of May were rainy. The summer of 2013 was warmer and drier than usual. Consequently, winter crop development was faster; they even ripened earlier than usual, at the end of July. Due to this rapid development yield was lower than in 2012.

Materials and methods used in bread baking tests

A hybrid variety of rye ‘Agronom’ and population variety of rye ‘Kaupo’ cultivated at the State Plant Breeding Institute Priekuli and State Cereals Breeding Institute Stende trial fields, were used in the research. Cleaned and prepared for processing, the grain samples were ground in a Hawos Queen2 mill at the Bread Manufacturing Laboratory of Latvia University of Agriculture, Faculty of Food Technology and whole meal rye flour with moisture content of 11.2 to 12% was obtained for bread baking.

The enzyme asparaginase (Megazyme) with activity: 15 U/mg (25 °C, pH 8.0), the natural antioxidant rosemary extract „Oleoresin Rosemary 41-19-25” (recommended amount of addition 0.08% to dough mass), and citric acid (E330) were used in the baking tests.

Whole meal rye bread bakery tests

Bread bakery tests were carried out to assess enzyme asparaginase, rosemary extract and citric acid impact on acrylamide formation intensity in rye bread samples, which were prepared in an industrial module system. Hybrid variety ‘Agronom’ and population variety ‘Kaupo’ whole meal rye flour, sugar, salt and water were used for the dough preparation. The dough recipe is shown in Table 1. The dough was prepared in accordance with bread making technology, including starter preparation, dough kneading and fermentation, dough dividing, loaf formation and baking.

<table>
<thead>
<tr>
<th>Table 1 Whole meal rye bread basic recipe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semi-finished product</td>
</tr>
<tr>
<td>----------------------------</td>
</tr>
<tr>
<td>Yeast bread</td>
</tr>
<tr>
<td>Dough</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

The starter was made using German Böcker pure rye bread starter culture. The starter was prepared separately for each flour sample, the necessary temperature (28–30 °C) and fermentation time (2×24 hours) were provided. 50% of the total quantity of rye flour was used for the starter. The starter acidity ranged from pH 4.0 to 3.9, which corresponds to the required acidity for rye bread starter.

The dough was mixed for 10 minutes until all the ingredients were evenly blended and a homogeneous mass resulted. Enzyme asparaginase (500 U) was added to four samples at the end of dough mixing. The dough fermented for 30 minutes, then was divided into 400 gram loaves, then put into forms and fermented for 60 minutes in the post-fermentation chamber at a temperature of 35 °C and 70% relative air humidity.
Fermented semi-finished dough was baked in a Sweba Dahlen convection oven at a temperature of 200 °C for 40 minutes. After baking, the bread samples were cooled and their taste was evaluated. The bread samples were baked for 50 minutes in the clay oven at a falling temperature regime (initial temperature 260 °C, final temperature 210 °C). The added rosemary extract Oleoresin Rosemary 41-19-25 proportion was 0.1% and 0.5%, and citric acid proportion was 1%, following the manufacturer's recommendations. The baking tests for rye bread with dried fruits, with and without the addition of rosemary extract (0.1%), were conducted at the bakery.

Amino acids in flour, dough and rye bread samples
Amino acids were analysed according to AOAC standard method N. 994.12, by using ion change after sample hydrolysis by 6M HCl in an inert atmosphere. The content of amino acids was determined by using an Automatic Amino Acids Analyzer (Microtechna Praha), and as the standard, the „Amino acid Standard solution for protein hydrolysates 0.5 µmoles per mL” (Sigma).

Acrylamide
Acrylamide separation from the bread samples was achieved with solid phase extraction, but the acrylamide content was analysed with liquid chromatograph-tandem mass spectrometry (LC-MS/MS). For analysis 2±0.0001 g of dried and homogeneous sample was weighed into a 50 mL centrifuge tube. The internal standard was added (d3-acrylamide) at 100 µg kg⁻¹. Then 5 mL of hexane was added for sample degreasing, and then the tube was vortexed. After 10 mL of water, 10 mL of acetonitrile, 4.0 g MgSO₄ and 0.5 g NaCl were added the tube was vigorously shaken for 1 min, then centrifuged at 4000 rpm for 5 min. The hexane layer was discarded and 1 mL of the acetonitrile extract was transferred to a tube containing 50 mg of primary and secondary amines (PSA) and 150 mg MgSO₄. The tube was vortexed for 30 sec and then placed in an autosampler vial, and analysed with LC-MS/MS (Waters 2695, Quattro Premire XE). The determination of acrylamide was performed at the Latvia Institute of Food Safety, Animal Health and Environment “BIOR”

Statistical analysis. Experimental data evaluation was done using two–factor analysis of variance by Fisher’s criteria and least significant difference (LSD₀₅). Components of variance ANOVA for each quality characteristic were expressed as percentages to illustrate the relative impact of each source to the total variance.

Results and Discussion
Field trials and rye cultivation
Hybrid rye variety ‘Agronom’ and population rye variety ‘Kaupo’ were grown in field trials. Scientific literature indicates that rye hybrid varieties generally are higher yield than rye population varieties (Petr, 2006). In this 2013 trial, such a difference was not observed. This may be due to the relatively rapid development of the plants in the summer of 2013, as a result hybrid rye have not realizing its potential for yield. The 2013 yield was influenced significantly by fungicide usage at sowing – both varieties have significantly higher yield with fungicide usage – more than one tonne per hectare (Table 2).

<table>
<thead>
<tr>
<th>Variety</th>
<th>NPK+R+H</th>
<th>NPK+R+H+F</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Agronom’ FI (hybrid)</td>
<td>5.62</td>
<td>6.65</td>
<td>6.13</td>
</tr>
<tr>
<td>‘Kaupo’ (population)</td>
<td>5.73</td>
<td>6.48</td>
<td>6.11</td>
</tr>
</tbody>
</table>

In 2013 there was no significant difference between the tested populations of hybrid varieties of rye and yields. The yield of both varieties of rye in the presence of fungicide increased. Samples were submitted from both varieties harvested and submitted for further analysis. The study locations were the State Plant Breeding Institute Priekuli (hereinafter referred to as Priekuli) and State Cereals Breeding Institute Stende (hereinafter referred to as Stende).

The soil agrochemical parameters

<table>
<thead>
<tr>
<th>Sample of soil</th>
<th>Reac.</th>
<th>Organic matter</th>
<th>P₂O₅</th>
<th>K₂O</th>
<th>S-SO₄</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH, HCl</td>
<td>%</td>
<td>mg kg⁻¹</td>
<td>mg kg⁻¹</td>
<td>mg kg⁻¹</td>
</tr>
<tr>
<td>Stende 1</td>
<td>5.4</td>
<td>1.7</td>
<td>63.0</td>
<td>121.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Stende 2</td>
<td>4.0</td>
<td>4.0</td>
<td>271.0</td>
<td>145.0</td>
<td>1.2</td>
</tr>
<tr>
<td>Stende 3</td>
<td>4.0</td>
<td>2.1</td>
<td>256.0</td>
<td>140.0</td>
<td>1.2</td>
</tr>
<tr>
<td>Priekuli 1</td>
<td>4.8</td>
<td>1.9</td>
<td>95.0</td>
<td>106.0</td>
<td>0.6</td>
</tr>
<tr>
<td>Priekuli 2</td>
<td>6.0</td>
<td>2.5</td>
<td>101.0</td>
<td>88.0</td>
<td>0.6</td>
</tr>
<tr>
<td>Priekuli 3</td>
<td>4.9</td>
<td>2.1</td>
<td>100.0</td>
<td>127.0</td>
<td>0.6</td>
</tr>
</tbody>
</table>

In both study sites the research was carried out on the soil of relatively poor sod-podzolico and sod-podzolico sandy loam. The agro-chemical characteristics of the soil were assessed in accordance with the March 15ᵗʰ, 2007, Ministry of Agriculture (Latvia’s) guideline No. 12 “Methodological procedures for agro-chemical soil exploration and evaluation of the results”, as outlined in Table 3. Stendes soil is characterized by medium acid to very acid reaction, from insufficient to elevated organic matter content, from low to very high phosphorus content, and medium calcium content. Priekuli soil is characterized by very to normal acid reaction, insufficient to optimal organic matter content, and low to medium calcium content. In all of the soil samples found in both Stende and Priekuli, sulphur content was very low. According to research results, the optimal (sulphate) sulphur content
of soil could be from 10 to 60 mg kg\(^{-1}\), depending on organic matter in the soil. However, soils poor in organic matter contain sulphur of less than 5 mg kg\(^{-1}\). One explanation might be the soil fertilization trend of recent years: natural manure appears to have been replaced with manufactured phosphorous fertilizers as the European Union and other countries try to enforce measures aimed at the reduction of sulphur emissions from industrial production processes (Smit et al., 2009). It should be noted that sulphate sulphur leaches out of soil. Results of the soil analysis show that there is a need to further study asparagine and aspartic acid content changes in rye which occur as a result of cereal growing technologies used, taking into account both fertilization systems and the agrochemical properties of agricultural lands.

**Amino acid content of rye flour**

Aspartic acid is an amino acid, its structure has one amino group and two carboxyl groups, but in reaction with free NH\(_3\) forms amide of aspartic acid, or asparagine, it is known as a neutral polar amino acid. The study identified 16 amino acids in rye flour.

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Amino acids of whole grain rye flour, g 100 g(^{-1})</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>Kaupo 1</th>
<th>Kaupo 2</th>
<th>Agronom 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valine</td>
<td>0.41</td>
<td>0.50</td>
<td>0.58</td>
</tr>
<tr>
<td>Leucine</td>
<td>0.66</td>
<td>0.75</td>
<td>0.82</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.37</td>
<td>0.41</td>
<td>0.46</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>0.98</td>
<td>1.08</td>
<td>1.22</td>
</tr>
<tr>
<td>Lysine</td>
<td>2.92</td>
<td>2.53</td>
<td>2.68</td>
</tr>
<tr>
<td>Arginine</td>
<td>0.49</td>
<td>–</td>
<td>0.52</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>0.65</td>
<td>0.79</td>
<td>0.90</td>
</tr>
<tr>
<td>Serine</td>
<td>0.43</td>
<td>0.46</td>
<td>0.53</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>2.29</td>
<td>2.65</td>
<td>3.02</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.49</td>
<td>0.54</td>
<td>0.58</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.31</td>
<td>0.35</td>
<td>0.39</td>
</tr>
<tr>
<td>Alanine</td>
<td>0.36</td>
<td>0.54</td>
<td>0.62</td>
</tr>
<tr>
<td>Proline</td>
<td>1.00</td>
<td>1.13</td>
<td>1.31</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.19</td>
<td>0.22</td>
<td>0.26</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.11</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Total</td>
<td>11.65</td>
<td>12.05</td>
<td>13.97</td>
</tr>
</tbody>
</table>

The amino acids content of rye flour is given in Table 4. The total content in ‘Kaupo’ variety rye whole meal flour was from 11.65 to 12.05 g 100 g\(^{-1}\) but in hybrid variety ‘Agronom’ was 13.97 g 100 g\(^{-1}\). The aspartic acid content of rye whole meal flour was from 0.65 to 0.9 g 100 g\(^{-1}\). Literature data shows that the aspartic acid content of whole grain rye is 503 µg kg\(^{-1}\) (Mustafa et al., 2007). This indicates that the flour used in the study has higher aspartic acid content.

Rye bread baking tests for evaluating enzyme asparaginase, rosemary extract and citric acid influence on acrylamide formation. To evaluate the impact of recipes and bread baking technology on acrylamide formation, the amino acid content was measured in dough and bread, as well as acrylamide content in the bread samples. Obtained results are given in Table 5.

| Table 5 | Acrylamide content in samples of different types of rye bread |

<table>
<thead>
<tr>
<th>No</th>
<th>Description of rye bread (rye grain variety, growing place)</th>
<th>Acrylamide content, µg kg(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rye bread baked in form (‘Kaupo’, Priekuli)</td>
<td>10.9</td>
</tr>
<tr>
<td>2</td>
<td>Rye bread baked in form (‘Kaupo’, Priekuli) with enzyme</td>
<td>14.9</td>
</tr>
<tr>
<td>3</td>
<td>Rye bread baked in form (Agronom, Priekuli)</td>
<td>17.4</td>
</tr>
<tr>
<td>4</td>
<td>Rye bread baked in form (‘Agronom’, Priekuli)</td>
<td>16.4</td>
</tr>
<tr>
<td>5</td>
<td>Rye bread baked in form (‘Agronom’, Stende)</td>
<td>12.5</td>
</tr>
<tr>
<td>6</td>
<td>Rye bread baked in form (‘Kaupo’, Stende) with enzyme</td>
<td>15.6</td>
</tr>
<tr>
<td>7</td>
<td>Rye bread baked in form (‘Kaupo’, Stende)</td>
<td>16.8</td>
</tr>
<tr>
<td>8</td>
<td>Rye bread baked in form (‘Agronom’, Stende) with enzyme</td>
<td>18.2</td>
</tr>
<tr>
<td>9</td>
<td>Rye bread (‘Kaupo’, Priekuli) control</td>
<td>98.6</td>
</tr>
<tr>
<td>10</td>
<td>Rye bread (‘Kaupo’, Priekuli) with Rosemarie extract 0.1%</td>
<td>100.7</td>
</tr>
<tr>
<td>11</td>
<td>Rye bread (‘Kaupo’, Priekuli) with citric acid 1%</td>
<td>34.3</td>
</tr>
<tr>
<td>12</td>
<td>Rye bread (‘Kaupo’, Priekuli) with Rosemarie extract 0.5%</td>
<td>106.6</td>
</tr>
<tr>
<td>13</td>
<td>Rye bread with fruits (at production company) control</td>
<td>177.1</td>
</tr>
<tr>
<td>14</td>
<td>Rye bread with fruits (at production company) with Rosemarie extract 0.1%</td>
<td>238.6</td>
</tr>
</tbody>
</table>

In analysing amino acid content in rye dough and bread, the most attention was heeded to aspartic acid content and its changes during bread production. The aspartic acid in rye dough samples was from 0.38 to 0.48 g 100 g\(^{-1}\). Significant distinctions between different samples and samples with or without adding enzyme asparaginase were not observed. The aspartic content of bread samples ranged from 0.32 to 0.84 g 100 g\(^{-1}\). However, it should be noted, the relation between different samples was not observed.

The enzyme asparaginase was added to bread samples, 500 U kg\(^{-1}\) in relation to flour weight. Scientific literature indicates that the amount of added enzyme can be from 200 to 2000 U kg\(^{-1}\), and can reduce acrylamide content up to 70% (Capuano, 2009). The bread samples baked in forms (Fig. 1) do not have dark and thick crusts, and the acrylamide content is from 10.9 to 18.2 µg kg\(^{-1}\). Such acrylamide content is
significant, according to the Joint Food and Drug Administration (FDA) and World Health Organization (WHO) Expert Committee on Food and Additives (JECFA). Their recommendation is that the acrylamide content in bread should not exceed 150 µg·kg⁻¹ (EFSA, 2009). The impact of enzyme action on changes in acrylamide content was not observed. Admittedly, the enzyme asparaginase is expensive, and its wider utilization in bread manufacturing is not cost effective. The evaluation of the bread quality and physical chemical indicators between different varieties of rye grains and different cultivation regions did not show significant differences. The moisture of all dough samples was similar, ranging from 48.5 to 50%. The moisture of the bread samples was from 40.2 to 41.6%.

The study evaluated the impact of a natural antioxidant and citric acid on acrylamide content and rye bread quality. The bread samples were baked on clay, resulting in a darker and thicker crust than in samples baked in forms.

Analysis of acrylamide content in bread samples baked on clay (Table 5) show that it has increased – from 100.7 to 106.6 µg kg⁻¹ in samples with added rosemary extract, and 98.6 µg kg⁻¹ in the control sample. From this it can be concluded that rosemary extract added to rye bread dough, in amounts of 0.1 and 0.5% does not have significant impact on acrylamide content. The rye bread with fruits, baked in an industrial bakery, has an acrylamide content of 177.1 µg kg⁻¹, which exceeds the recommended quantity, but the addition of 0.1% rosemary extract raises acrylamide content up to 238.6 µg kg⁻¹.

The research carried out by Ozolina (2011) concluded that bread type, baking oven, and sugar content in dough do not have significant impact on acrylamide content in bread, but that the addition of dried fruits significantly impacts the content of acrylamide in the bread (p=0.025). The sugar content is not the main reason for the presence of acrylamide in bread, as also confirmed by a similar study (Springer et al., 2003). The scientific literature has no information about the possible influence of dried fruit additives on the acrylamide content in bread and other products.

The bread samples with 1% citric acid showed an acrylamide decrease of 66%, and in the bread it is 34.3 µg kg⁻¹. It should be noted that the sensory qualities of bread with such a large citric acid presence were lower than that of control samples because the bread taste was markedly sour. These results suggest that the presence of citric acid can reduce acrylamide content in bread, only the quantity to be added must be correctly chosen, in order to not decrease the product sensory characteristics.

Based on the acrylamide content in foodstuff, it is possible to calculate contamination intake level in the human body. If daily ingestion of whole grain rye bread is 200 g, then acrylamide intake is 9 to 10 µg. Thus the calculation for a person weighing 70 kg, is approximately 0.13 µg acrylamide per kg body weight.

**Figure 1. Samples of rye bread baked in forms**

According to World Health Organization information, the level of acrylamide intake from the Latvian bread used in this case is medial. Although this indicator is not high, the issue of acrylamide content and the means to reduce it are actually urgent.

**Conclusions**

The results of soil composition analysis indicated insufficient content of sulphur containing compounds, which significantly impacted aspartic acid and asparagine content in grains as well as on bread quality.

The results of soil analysis give evidence for further investigations necessary on asparagines and aspartic acid changes in rye in connection with technology used in cereals cultivation in Latvia, with evaluation on fertilizer systems as well as agricultural land agrochemical properties.

Baking time significantly influenced the formation of acrylamide, therefore it is critical that the optimum baking time for specific bread types is not exceeded. The results confirm the studies of other researchers who have observed that acrylamide content in bread increases with prolonged baking time and increased temperature. The use of the enzyme asparaginase in the production of rye bread does not significantly reduce acrylamide content.

The addition of rosemary extract to rye dough up to 0.5% does not reduce the content of acrylamide in rye bread.

The addition of citric acid (1%) to rye dough reduced the acrylamide content in bread by 66%. It is necessary to evaluate the admissible amount of citric acid in order to not change the bread sensory qualities of acidity and aroma.

Acrylamide content in rye bread significantly increases with the introduction of dried fruit into the bread.

The reduction of acrylamide content in bread should be treated as a complex system of interlinked factors: soil, grains, technological processing and bread.

**References**

QUALITY CHANGES OF CEREAL MUESLI WITH SEEDS DURING STORAGE
Santa Senhofa, Evita Straumite, Dace Klava

Abstract
Cereals play a vital role in diet because they not only provide humans with essential macronutrients such as protein, fat and carbohydrate for growth and maintenance but also supply with vitamins, minerals, and micronutrients for optimal health. In recent years the activities of human consumption “clean” products, with no extra added sugars, food additives and preservatives increases. The aim of this study was to evaluate cereal muesli with seeds quality changes during storage. Samples of muesli with seeds (linseeds, pumpkins seeds, almond slices) were packaged in 3 different packages – paper bag, paper tubes and Doypack (standup pouches) and stored for 6 months (at 20±2 °C temperature and air humidity – 50±5%). During the storage moisture content, water activity, and microbiological safety were evaluated.

After 6 months storage of muesli with seeds, the moisture content in samples packaged in the paper tube decreases by 14.3%, in Doypack –20.5% and in paper bag by 34.9%. The lowest moisture content was determined for muesli samples storage in the paper package – 6.00%. Water activity compared to the fresh prepared muesli (a_w=0.56) decreased, in paper tube it was 0.33, in paper bag – 0.16 and in Doypack – 0.47. As more unusable for cereal muesli with seeds packaging paper bags was detected, because of essential quality changes of samples during storage. After 6 months storage total count of microorganisms and moulds increase, but remained within the normal range. Therefore, the shelf-life of cereal muesli with seeds packaged in paper tube or Doypack for 6 months could be recommendable.

Keywords: cereals, muesli, packaging, storage time.

Introduction
Muesli is a mixture of grain flakes and dried fruits, where can be also added seeds and nuts. Thanks to its easy and quick preparation with milk, yogurt or hot water, muesli is often called as a breakfast flakes. Cereals play a vital role in diet because they are not only providing humans with essential macronutrients such as protein, fat and carbohydrate for growth and maintenance but also supply other important vitamins, minerals, and micronutrients for optimal health (Collins et al., 2010). The breakfast cereals are generally high in carbohydrate are low in fat; some have high bran contents, and many contain appreciable amounts of certain vitamins and minerals, partially satisfying the mineral and vitamin requirements (Albertson et al., 2008; Bertais et al., 2000). Eating cereal at breakfast was associated with increased consumption of fibre and carbohydrates and decreased consumption of fats throughout the day (Gibson, Gunn, 2011; Albertson et al. 2008; Williams, 2007; Wilson et al., 2006), and improved mental and greater physical performance (Albertson et al. 2008; Rampersaud et al., 2005). This may be because they are a good source of whole grains and commonly eaten with milk. Whole grains are rich in dietary fibre and both grains and milk will improve micronutrient intake (Coudray, 2011; Drewnowski, 2011; Vissers et al., 2011; Williamson, 2010). Breakfast cereals are often now also fortified with vitamins such as folate and thiamine (Loui et al., 2012; Hannon et al., 2007; Berner et al., 2001). Cereal consumption as part of a healthy lifestyle may play a role in maintaining adequate nutrient intake and physical activity (Albertson et al., 2008).

The earliest breakfast cereal manufacture was based on boiling then drying, extrusion has become a well-established industrial technology with beneficial effects on the nutritional properties and texture of the raw material. The pleasant flavours and colours of breakfast cereals are produced in the drying and toasting steps (Delgado-Andrade et al., 2007; Rufian-Henares et al., 2006; Rada-Mendoza et al., 2004). The majority of cereal-associated bacteria are located in the outer layers of the kernel and are thus concentrated in bran (ICMFS (International Commission on Microbiological Specifications for Foods), 2005; Laca et al., 2006). Contamination of the grains during storage, transport, and processing further affects the microflora.

Texture in ready-to-eat cereals is fundamental for product acceptance by consumers. Textural properties of food are used by consumers as key quality indicators that contribute to product acceptability. Crispness is one such key indicator and is considered a primary textural attribute of breakfast cereal. Cereal hardness is another force claimed that crispness is the most important attribute affecting consumer acceptability (Pathare et al., 2012; Burrington, 2001). Moisture content for breakfast cereal is as the critical quality parameter and relative humidity as the most influential environmental factor (Macedo et al., 2011).

The use of packaging in the food supply chain is very important and is an essential part of food processing. It can be used for various purposes such as the assurance of protection of food from external infections (microorganisms), surrounding conditions (like atmosphere gases, water activity, etc.), and the appropriate labelling of foods (La Storia et al., 2008). Flexible pouch used to package breakfast cereal in air is not integral to the product. The cereal could be removed from the package and poured into a plastic dispenser and not suffer any loss in shelf life. Cereal products are usually packaged in paper/polyethylene
Results and Discussion

Moisture content

During 6 months of storage, muesli moisture content in paper tube decreased by 14.3%, in paper bag with window – by 34.9% and in Doypack by 20.5% (Figure 1.). Muesli moisture varied from 9.2% at first to 6.0% at the end.

![Figure 1. Changes in moisture content of muesli with seeds during storage](image)

The lowest moisture content was determined in the paper bag with PP window after 6 month storage – 6.00%. Legislation lay down that moisture content for flakes has to be in the range between 10–14% (Prasības..., 2008). Comparing obtained results with other researches, results shows, that oat flakes moisture content is between 6.0–8.7% (Gates et. al., 2007).

In the paper bag with PP window it has decreased more than in the paper tube and Doypack. This difference can be due to the quality of the packaging material. High moisture content may be caused to the additives and their interaction with each other by connecting a number of components. Each additive moisture content is variable and storing the environment constitute the final product which resulted on the product quality. During storage, the product has seen a steady, slow decline of moisture, which can be related to the raw materials used in the product, which has the ability to bind moisture from one to another by connecting to creating a favourable environment for microbial growth.

Water activity (a_w)

Water activity (a_w) was used to determine stable shelf-life for foods. During storage of the muesli a decrease in water activity was observed (Fig. 2.). Water activity for muesli with seeds ranged between 0.56 at first to 0.16 at the end. These differences affected packaging materials – the lowest water activity was determined in the paper bag – 0.16. In the paper tube water activity (a_w) decreased for 41.1% and in Doypack – for 16.1%. Water activity for muesli products is not defined in legislation, but there are some researches, where water activity for dried foods is

Characterisation of packaging materials

Samples of muesli with seeds (linseeds, pumpkins seeds, almond slices) were packaged in 3 different packaging materials:
- paper tubes – tubes with aluminium layer inside and LDPE (low density polyethylene) black cover;
- paper bag – brown kraft paper 90–100 g m⁻² with PP (polypropylene) window;
- Doypack (standup pouches) – Pap50g/Alu7/Pe60.

Amount of sample in package – 250 g, storage time 6 months at 20±2 °C temperature and air humidity – 50±5%.

Moisture content

Moisture content of muesli with seeds was determined using heating oven Memmert (GmbH) Memmert, Germany – 5.00±0.03 g sample for 1 hour was dried at 110±1 °C temperature (LVS EN ISO 712:2010A). In the analysis on moisture content was run in duplicate and averaged.

Water activity (a_w)

Water activity (a_w) was determined using LabSwift-aw (AG Novasina, Switzerland) equipment. In the analysis on water activity analysis was run in triplicate and averaged.

Microbiological parameters

Total plate count of mesophilic aerobic and facultative anaerobic microorganisms (MAFAm) were determined in conformity with the standard LVS EN 4833:2003; yeasts and moulds – with the standard ISO 21527-2:2008.

Statistical analysis

The mean and standard deviation were processed by mathematical and statistical methods.

Materials and Methods

Characterisation of muesli with seeds

Muesli with seeds contains:
- 40% roasted whole grain triticale flakes (200 °C for 10 min),
- 25% roasted whole grain rye flakes (200 °C for 10 min),
- 15% linseeds,
- 10% almond pieces,
- 10% pumpkin seeds.

Characterisation of packaging materials

The mean and standard deviation were processed by mathematical and statistical methods.

Microbiological parameters

Total plate count of mesophilic aerobic and facultative anaerobic microorganisms (MAFAm) were determined in conformity with the standard LVS EN 4833:2003; yeasts and moulds – with the standard ISO 21527-2:2008.

Statistical analysis

The mean and standard deviation were processed by mathematical and statistical methods.

Moisture content

During 6 months of storage, muesli moisture content in paper tube decreased by 14.3%, in paper bag with window – by 34.9% and in Doypack by 20.5% (Figure 1.). Muesli moisture varied from 9.2% at first to 6.0% at the end.

![Figure 1. Changes in moisture content of muesli with seeds during storage](image)

The lowest moisture content was determined in the paper bag with PP window after 6 month storage – 6.00%. Legislation lay down that moisture content for flakes has to be in the range between 10–14% (Prasības..., 2008). Comparing obtained results with other researches, results shows, that oat flakes moisture content is between 6.0–8.7% (Gates et. al., 2007).

In the paper bag with PP window it has decreased more than in the paper tube and Doypack. This difference can be due to the quality of the packaging material. High moisture content may be caused to the additives and their interaction with each other by connecting a number of components. Each additive moisture content is variable and storing the environment constitute the final product which resulted on the product quality. During storage, the product has seen a steady, slow decline of moisture, which can be related to the raw materials used in the product, which has the ability to bind moisture from one to another by connecting to creating a favourable environment for microbial growth.

Water activity (a_w)

Water activity was used to determine stable shelf-life for foods. During storage of the muesli a decrease in water activity was observed (Fig. 2.). Water activity for muesli with seeds ranged between 0.56 at first to 0.16 at the end. These differences affected packaging materials – the lowest water activity was determined in the paper bag – 0.16. In the paper tube water activity (a_w) decreased for 41.1% and in Doypack – for 16.1%. Water activity for muesli products is not defined in legislation, but there are some researches, where water activity for dried foods is
Reduced water activity does not provide a complete inactivation of microorganisms, but partially restrict their activities in the product, which helps to ensure a longer shelf-life. Having a water activity below 0.6 products are stable against microbial growth, but chemical and enzymatic reactions can occur which result in deterioration. Comparing data with other research, it can be concluded that the muesli stored up to 12 months, the water activity is 0.22 (Labuza, 1980).

**Microbiological parameters**

Taking into account that the grain seed mixture contains more different components that are re-cooked as they are received from a supplier, the product is increased microbial risk.

![Figure 2. Changes in water activity (a_w) of muesli with seeds during storage](image)

1 – paper tube, 2 – paper bag, 3 – Doypack

**Conclusions**

The shelf-life of cereal muesli with seeds packaged in paper tube with aluminium layer inside and LDPE black cover or Doypack (stand-up pouches) Pap50g/Alu7/Pe60 for 6 months could be recommendable. The shelf-life of cereal muesli with seeds packaged in paper bag with PP window for 4 months could be recommendable.

**Acknowledgment**

The authors are grateful to Alise Balgalve (SIA Felici), who has contributed to the studies with advice, suggestions and help with research materials.

**References**


APPLICATION OF ENZYMATIC TREATMENT TO IMPROVE THE CONCENTRATION OF BIOACTIVE COMPOUNDS AND ANTIOXIDANT POTENTIAL OF WHEAT AND RYE BRAN

Vitalijs Radenkovs1,2, Dace Klava2, Inta Krasnova1, Karina Juhnevica-Radenkova1

1 Latvia State Institute of Fruit-Growing, 1 Grauda street, Dobele, Latvia, e-mail: vitalijs.rad@inbox.lv
2 Department of Food Technology, Faculty of Food Technology, Latvia University of Agriculture, 2 Liela street, Jelgava, Latvia

Abstract
The present study was undertaken to establish the effect of enzymatic treatment on the content of total phenolic compounds and antioxidant activity in enzymatically treated bran. Enzymatic hydrolysis of bran was carried out by α-amylase from Bacillus amyloliquefaciens (Sigma Aldrich) for breakdown the bonds between glucose monomers in starch. Multi enzyme complex (Viscozyme L) containing a wide range of carbohydrates were used for depolymerisation of cellulose and hemicelluloses molecules. The 80% ethanol was used to extract the antioxidant compounds from bran. Free radical scavenging activity of samples was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH). Assay and the data were expressed in Trolox equivalents (TE) per 100 g1 of sample, as well as the reducing power was determined using ferric reducing antioxidant power (FRAP) assay and the data were expressed in the same indices. The obtained results showed that the enzymatically treated bran samples had the highest concentration of total phenolic compounds, on the other hand the enzymatically treated bran showed higher antioxidant potential than non-enzymatically treated bran samples. Extract from enzymatically treated rye bran had the highest concentration of phenolic compounds, 1230±42.57 mg GAE 100 g−1 DW. The lowest concentration of phenolic compounds was found in untreated wheat bran samples and this amount was equal to 377±9.78 mg GAE 100 g−1 DW. Two different methods of evaluation of the bran antioxidant activity showed potential usefulness of enzymatic treatment.

Keywords: phenolic compounds, antioxidant activity, bran, enzymatic hydrolysis.

Introduction
Bran of wheat and rye is a by-product from the milling process of flour and is a composite material formed from different histological layers, and three different strips. The outer strip corresponds to outer pericarp (epidermis and hypodermis), the inner one corresponds to the aleurone layers, and the intermediate one remains a composite of several tissues (inner pericarp, testa, and nuclear tissue (Hemery et al., 2010). Phenolic compounds derived from whole grain as well as from grain fractions have health-promoting effect. In the plant kingdom, phenolic compounds are essential molecules against oxidative damage, as they have UV-absorption properties and radical-scavenging activities. Therefore, the majority of the phenolic compounds are located in the most external tissues of the plant (Liu et al., 1995). In wheat grain, most of the phenolic compounds are located in the bran, which constitutes the outermost part of the grain. One of the milling functions is cereal gain dehulling and debranning with the aim to obtain the white flour without any impurities.

Cereal grains and especially outer parts of the cereal are a good source of phenolic compounds. In cereal grains located considerable amount of bioactive compounds e.g. phenolic acids, saponins, while flavonoids and phytoestrogens are presented in small quantities (Dordević et al., 2010). Scientific works imply that phenolic compounds have relatively high antioxidant activity, which may promote to their health benefits. The most predominant phenolic compound in cereals is ferulic acid, which forms up to 90% of total polyphenols. Other phenolic acids like p-coumaric, m-coumaric, syringic and vanilic acid have also been reported in cereals (Hosseinian, Mazza 2009).

Grain as well as bran chemical composition including phenolic compounds mostly depends from grain genetic / agricultural backgrounds, growing conditions and storage. On the other hand strong effect on chemical composition renders the milling procedure (Adom et al., 2005). Liyana-Pathirana and Shahidi (2006) reported that the contribution of bound phenolics to the total phenolic content in wheat was significantly higher than free and esterified fractions, and the bound phenolic fraction demonstrated a significantly higher antioxidant capacity than free and esterified phenolics. Li et al. (2010) have reported that the phenolic compounds in cereals were mostly found in three forms: insoluble (66–80%), soluble conjugated (17–30%) and free phenolics (6%). The covalently bound ferulic acids during the fermentation of wheat bran fiber in a human model colon were released (Kroon et al., 1997). Although the solvent extraction is the major method to extract bioactive compounds from plant materials, or to obtain plant extracts rich in bioactive compounds. In the world science there have been several contentious moments e.g. after the extraction of bioactive compounds by using different types of solvents the components have low recovery and strict regulations for the use of these kind of products in the food industry. Enzymatic hydrolysis is one of the extraction techniques without any organic solvents and toxic chemicals that gave positive result and advantages among other conventional procedures. Main mechanisms of enzymatic hydrolysis are convert water-insoluble components into water soluble materials (Athukorala et al., 2006). For example, Heo et al. (2005) reported that enzymatic hydrolysis of brown seaweeds gained high bioactive compound yield and showed enhanced biological activity compared
Materials and Methods

Experiments were done at the Latvia State Institute of Fruit – Growing collaboration with Riga Technical University.

Chemicals

Ethanol (96%) was received from SIA Jaunpagasts Plus (Company Jaunpagasts Plus Ltd., Latvia). Methanol, ethanol, ethyl acetate, asodium chloride (NaCl), hydrochloric acid (HCl), Folin-Ciocalteu reagent were purchased from Sigma-Aldrich (Steinheim, Germany).

Bran samples

Summer wheat (Triticum aestivum) and rye (Secale cereale) bran samples were collected from industrial mills in Latvia:

1. SC Dobeles dzirnavnieks – small particle size wheat bran (WSSD);
2. SC Dobeles dzirnavnieks – wheat bran with large particle size bran (WLSD);
3. SC Rigas dzirnavnieks – large particle size wheat bran (WLSR);
4. SC Jelgavas dzirnavnieks – small particle size rye bran (RSSJ).

Enzymes

Industrial enzymes preparations produced by “Novozyme Corporation” (Bagsvaerd, Denmark) and purchased from Sigma-Aldrich. Two commercial preparations of enzymes: α-amylase from Bacillus amyloliquefaciens and Viscozyme L from Aspergillus spp., were used to hydrolyze carbohydrates. α-amylase has a declared activity ≥250 units g⁻¹, optimum conditions of enzymatic pretreatment is pH 5.0–8.0, temperature 55±1 °C and incubation time 0.5 h (Demirkan et al., 2004) form Viscozyme L declared activity is 100 FBG g⁻¹, optimum conditions are pH 4.6, temperature 44±1 °C and incubation time 3.2 h. In this scientific work enzymes were tested both independently and in combination for establishing the synergetic interaction.

Enzymatic Hydrolysis

For α-amylases treatments, wheat bran (10 g) was mixed with 90 mL of distilled water in 1000 mL. Reagent bottle with screw cap with dilutions 1 : 9, and then 500 µL of α-amylase was added. Hydrolysis was carried out in a water bath at temperature 55±1 °C, incubation time 0.5 h and shaking intensity 60 rpm. After starch hydrolysis and enzyme inactivation (10 min temperature 100±1 °C) wheat bran mash was 3 minutes homogenized, the pH of the suspension was adjusted to pH 4.6 with 0.2 mL 50% citric acid in each dilutes and Viscozyme L 400 µL was added. Incubation time is 3.2 h, temperature 44±1 °C, and shaking intensity 60 min⁻¹.

Extraction of Phenolic acids

The free phenolic acids were isolated using the procedure explained by Wang et al. (2006) with slight modification, and soluble conjugated phenolic compounds were isolated using procedure described by Robbins (2003) and is depicted Figure 1.

Figure 1. Flow diagram of extraction procedure of phenolic compounds

After free phenolic compounds extraction (×3 times) with 80% ethanol, after supernatant collection and the alkaline hydrolysis with 2 N NaOH was done. Alkaline hydrolysis was carried out at room temperature for 4 h, and after the hydrolysis the alkaline extract was neutralised with 6 M HCl. For elimination of precipitation the filtration through Watman paper filter No. 1 was done. The antioxidant activity and total phenolic compounds was immediately analyzed.
Determination of total phenolic compounds

Determination of total antioxidants reducing capacity by using Folin-Ciocalteu reagent was carried out as described by Sacchetti et al. (2009) with minor modifications. To volumetric flasks (10 mL) were pipette 100 μL of analyzed sample, 5 mL of deionized water and 0.5 mL of Folin reagent. After 3 minutes was added 1 mL of saturated sodium carbonate solution and supplemented with deionized water to 10 mL. The mixture was incubated for one hour at 23±2 °C, under dark, and then measured the absorbption on wavelength λ=765 nm. The results obtained were expressed as mg gallic acid equivalent (GAE) per g dry weight (DW).

Free Radical Scavenging Activity

Free radical scavenging activity of samples was measured using the 2,2-difeny1-1-picrylhazyl (DPPH) according to the procedures described by Yen, Chen (1995) with slight modification. The extracts (100 μL) were reacted with 2.9 mL of DPPH solution (0.0039 g DPPH in 100 mL methanol). Absorbance of the cereal extracts was determined using UV – Visible Spectrophotometer SHIMADZU at 515 nm. Free radical scavenging activity of the samples was expressed as mg Trolox equivalent antioxidant capacity per 100 g dry weight (mg TEAC g DW).

Free reducing antioxidant power (FRAP)

Free reducing antioxidant power (FRAP) was determined by its ability to reduce ferric to ferrous ions. When iron is complexed with 2, 4, 6-tripyridyl-st-trazine (TPTZ) in sodium acetate solution at an acidic pH, its reduction results in a color change of the solution, from pale rust to blue. The absorbance of the solution at 593 nm reflects the extent of reduction. The reduction power was expressed as mg Trolox equivalent. The extracts (100 μL) were reacted with 3.6 mL FRAP reagent and after vortex the absorption was spectrophotometrically detected.

Statistical analysis

Data was processed by SPSS software version 17.0. Data was analysed using descriptive statistics and processed by one-way analysis of variance ANOVA (one way ANOVA), as well as for comparing all bran samples depending from pre-treatment were used (two way ANOVA). Microsoft office software version 2007 was used to determine significant differences between the samples.

Results and Discussion

The plant cell wall is a complex design of polysaccharides. For the complete hydrolysis of these polysaccharides, a battery of enzymes is needed. By specific enzymatic treatments, cell wall polymer properties can be altered which can be utilised in food processing. Many plant cell walls contain phenolic acids residues that are ester-linked to the polysaccharide network. In grasses and cereals, these phenolic compounds (hydroxycinnamic acids) are mainly found esterified to arabinoxylans (5-O-feruloyl group). In decotyledons, such as spinach and sugar beet, ferulic acid is esterified to O-2 or O-3 position of arabinose and to O-6 position of galactose residues in pectin (Raeal et al., 1994; Fry, 1982).

The main activity of the Viscozyme L enzyme preparation is endo- (EK 3.2.1.4), exo-xylanases (EK 3.2.1.74), endo- (EC 3.2.1.4), exo-glucanase (EC 3.2.1.91) and β-glucosidase (EC 3.2.1.21). Endo-glucanase and exo-glucanase are known to act synergistically in cellulose hydrolysis (Wood, McRae, 1978) while β-glucosidase is needed for hydrolysing celllobiose (Woodward, Wiseman, 1982). Feruloyl esterase (FAE; EC. 3.1.1.72), are sometimes called hemicellulase accessory enzymes, subclass of the carboxylic acid esterases (E.C. 3.1.1.1). They splits the ester bond between the glycosides and hydroxycinnamic acids, which are preddented in the plant cell wall (Williamson et al., 1998). The esterases act to enable and facilitate the access of glycosyl hydrolases to the backbone wall polymers. Most feruloyl esterases act synergistically with xylanases, cellulases or pectinases to breakdown complex plant cell wall carbohydrates (Faulds, Williamson, 1995; Kroon, Williamson, 1996). Investigation into the effect of enzymatic treatments, on the content of bioactive compounds of the wheat and rye bran, revealed that treatments had a significant effect on the content of phenolic compounds, anthocyanins, as well as antioxidant potential. The influence of enzymatic treatments on the chemical compositions of the wheat and rye bran products indicated that the use of enzymes yielded a higher concentration of bioactive compound, than the untreated bran (Figure 2).

Figure 2. Concentration of total phenolic compounds in wheat and rye bran

The highest concentration of total phenolic compounds were found in enzymatically treated rye bran sample (RSSJ), and this amount was equal to 1230±42.57 mg GAE 100 g DM, followed by (WLSR) – 765.2±1.65 mg GAE 100 g DW, (WSSD) – 730.8±13.05 mg GAE 100 g DW, and (WLSR) – 541.9±3.41 mg GAE 100 g DW respectively. While the concentration of total phenolic compounds in untreated rye bran sample (RSSJ) were 520.7±7.17 mg GAE 100 g DM, followed by (WSSD), (WLSR), 461.3±17.16, 377.4±0.41, 377.0±9.78 mg GAE 100 g DW, respectively. The similar data was reported by Sungsopha (2009), which implies that after enzymatic treatments of rice bran the total phenolic compounds was increased by
476%, from 223.16 mg GAE 100 g$^{-1}$ DW to 836.21 mg GAE 100 g$^{-1}$ DW, and after author was made some conclusions, that it’s due to the effect that of enzymatic hydrolysis, liberates and frees phenolic components and increases the level of total phenolic compounds. Khoddami et al. (2013) was reported that bran treated with carbohydrases is significantly higher compared to untreated bran. Highest increase in vanillic and caffeic acids relative to the untreated bran was achieved by cellulase (3.7-fold) and Viscozyme (4.4-fold). The total content of free and bound phenolic acids was 668.5 μg g$^{-1}$ for untreated oat bran and after enzymatic treatments with Viscozyme L this amount has increased to 1116.0 μg g$^{-1}$.

**Microstructure of bran**
Wheat and rye bran is a multilayered composite, comprising a range of tissues, including the pericarp (epidermis, hypodermis, cross and tube cells) with the attached seed coat, the mucular epidermis, the aleurone layer, and remnants of the starchy endosperm. All of these tissues are dietary fiber with very low bioactive compounds and antioxidant bioaccessibility and bioavailability. Research concerning the bioaccessibility of phenolic compounds and other antioxidants from solid matrices are important, since only the compounds released from the food matrix and/or absorbed in the small intestine are potentially bioavailable and in a condition to exert their beneficial effects (Tagliazucchi et al., 2009). Phenolic compounds bound to dietary fiber need to be hydrolyzed by specific enzymes in the upper area of the intestine; otherwise, these compounds will not be bioaccessible for absorption in the human intestine but will be susceptible to degradation by the colonic microflora in the large intestine (Perez et al., 2009). Our study imply that the using of enzymes gives the possibilities release the bound form of phenolic compounds which increase the bioavailability of these material. Microscopy of the bran samples showed that degradation of cell walls was initiated in the pericarp layer (Figure 3B), as well in the starch/protein matrix (Figure 3C).

**Figure 3. Microstruture of bran obtained by SEM**

A–untreated wheat bran; B–enzymatically treated wheat bran (pericarp degradation), C–enzymatically treated wheat bran (starch/protein matrix degradation)

During the enzymatic hydrolysis the degradation of cell walls were observed by scanning electron microscope (Figure 3). Enzyme aided hydrolysation had large effects on wheat and rye bran characteristics, and partial hydrolysis of cell wall components was reflected in altered bran microstructure.

**Free Radical Scavenging Activity**
DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reductions capability of DPPH is determined by the decrease in its absorbance induced by antioxidant. In this study, the total antioxidant capacity of wheat and rye bran treated by using enzymes significantly increased (p<0.05) the number of equivalents of all the antioxidant standards. Analyzing data of free radical scavenging activity was detected only one exception for WLSR bran sample. After enzymatic treatment antioxidant capacity of this sample decreased from 18.35±0.70 to 15.84±0.59 mmol Trolox 100 g$^{-1}$ (Figure 4). It was useful to know that Folin-Ciocalteau assay give the possibility estimate not only phenolic compounds but also amino acids, carbohydrates, ascorbic acid, and other components which may increase the antioxidant activity of the samples. How it was reported in Kim and Wampler work that determination of total phenolic compounds by chemical method Folin-Ciocalteau assay gives the higher value than using instrumental HPLC method. There was reported that two methods are different, with different advantages and disadvantages (Kim, Wampler, 2011). Another author was reported that Folin-Ciocalteau assay gives a crude estimate of the total phenolic compounds present in an extract, whereas the free radical scavenging assay is not only specific to polyphenols (Prior et al., 2005). Our other work which at the moment not published suggest that during the extraction using different types of solvents as well as different techniques give opportunity extract from plant materials not only phenolic compounds but also some another components, which can interact with Folin-Ciocalteau reagent and simultaneously providing incorrect results. The highest scavenging effect of bran extracts on DPPH radical was observed in enzymatically treated bran (Figure 4).

**Figure 4. Free radical scavenging activity of bran extracts expressed as mg Trolox equivalent antioxidant capacity per 100 g$^{-1}$ of dry weight**

The highest scavenging effect was recorded in RSSJ bran sample (22.03±0.49 mmol Trolox 100 g$^{-1}$) followed by WLSR (15.84±0.59 mmol Trolox 100 g$^{-1}$), WLSD (15.63±0.53 mmol Trolox 100 g$^{-1}$), and WSSD.
(15.06±0.32 mmol Trolox 100 g\(^{-1}\)). The Sungsopha was reported similar results obtained during enzymatic hydrolysis of rice bran. After enzymatic treatments the antioxidant capacity was significantly increased (Sungsopha et al., 2009).

The antioxidant power of bran extracts was evaluated by FRAP assay in Figure 5.

In the present study, correlation graphs were plotted between IC50 values (including of DPPH, FRAP) and total phenolic contents. Two typical correlation graphs (i.e., DPPH vs total phenolic, FRAP vs total phenolic) are shown in Figure 6.

Several studies have attempted to correlate the DPPH scavenging activity of molecules to total phenolic content and to individual phenolic acids. In that respect Li, Wu, and Huang (2009) found that there was no direct correlation between DPPH inhibitory activity and total phenols, ferulic or caffeic acid contents of Radix angelicae sinensis, although they reported a correlation with 1/IC50 values (Li et al., 2009).

Another authors Gamel, Abdel-Aal (2012) were reported that total phenolic contents of barley samples correlated with DPPH inhibitory capacity. Verardo et al. (2011) found a strong correlation (R\(^2\)=0.93) of DPPH result of five oat cultivars to total free phenolic compounds. In this study the correlation (R\(^2\)=0.325) between DPPH and total phenolic compounds for the enzymatically treated wheat and rye bran is much weaker. The obtained results are incomparable due to fact that during the extraction of phenolic compounds was used different extraction techniques, which can effect on the extraction capacity.

**Conclusions**

This study suggest, that enzymatic treatment of wheat and rye bran are effective methods to improve the concentration of bioactive compounds and antioxidant activity. Therefore, these bran products may be exploited as a potent source of bioactive compounds and antioxidants, for nutraceutical and functional food products.

**References**

INVESTIGATION OF TOTAL DIETARY FIBER, B1 AND B2 VITAMIN CONTENT OF FLOUR BLEND FOR PASTA PRODUCTION

Solvita Kalnina, Tatjana Rakecieva, Ilze Gramatina, Daiga Kunkulberga

Department of Food Technology, Faculty of Food Technology, Latvia University of Agriculture, Liela Street 2, Jelgava, Latvia, e-mail: solvita.kalnina@inbox.lv

Abstract
The main purpose of the current research was to investigate total dietary fiber and vitamin B1 and B2 content in flour blends made from several types' whole grain flour for pasta production. In 2012 harvested convectional rye ‘Kaupo’, wheat ‘Zentos’, hull-less barley line ‘PR 5099’ and triticale line ‘940523’ grain and, for flour blends obtained, wheat flour type 550 was used in the experiments. Following quality parameters of flours using standard methods was evaluated: total dietary fiber content (ISO 5498) and vitamins B1 and B2 – by AOAC 986.27 and 970.65 respectively. In the present research significant differences in total dietary fiber of analysed whole grain rye, hull-less barley, triticale and wheat flour samples were established. Higher dietary fiber content was found in whole grain hull-less barley and rye flour, the lowest in the wheat flour type 550. Lower dietary fiber content was obtained for whole wheat and triticale flour blends. However, the higher, for whole rye and hull-less barley flour blend. B1 vitamin content in whole grain rye and wheat flour was significantly higher than in hull-less barley and triticale. Lowest vitamin B2 content was obtained in whole flour type 550. Lower B1 vitamin content, as 2.70±0.14 mg kg⁻¹ was obtained for whole hull-less barley flour blend, the higher as 3.19±0.28 mg kg⁻¹ for whole wheat flour blend. Lower B2 vitamin content, as 0.91±0.06 mg kg⁻¹, was obtained for whole hull-less barley flour blend, however, the higher B2, as 0.81±0.28 mg kg⁻¹ in whole wheat flour blend.

Keywords: grain, vitamins, dietary fibre, pasta, flour blends.

Introduction
Cereals and their products constitute an important part of the human diet, providing a high proportion of carbohydrates, proteins, fats, dietary fibres, B group vitamins and minerals (Okarter et al., 2010). Whole grains include cereal grains that consist of the intact, or ground, cracked or flaked fruit of the grains whose principal components are present in the same proportions as they exist in the intact grain containing a higher fiber, minerals and antioxidants (Slavin, 2004; Franz et al., 2006; Liu, 2007). Consumption of whole grain foods has been associated with decreased risk of cardiovascular disease and certain cancers, favourable effects on blood lipids and glucose, improved insulin resistance, and higher intakes of dietary fiber and micronutrients (McKeown et al., 2002). However, an understanding of optimal whole grain consumption levels, as well as the mechanisms by which whole grain foods exert their favourable effect’s is unclear (Lang et al., 2003; Slavin, 2003). Health benefits are associated with an increased intake of dietary fiber, including a reduced risk of coronary heart disease, diabetes, obesity, and some forms of cancer, as well as a reduction in cholesterol and fat (Anderson et al., 2009a; Brownlee, 2011).

Dietary fiber has an outstanding application as one of the key nutritional factors in the frame of a healthy diet. Dietary fiber remains a nutritional concept and comprises the edible part that escapes digestion in the small intestine and passes into the large intestine intact (Anderson et al., 2009a,b; Brownlee, 2011). Dietary fiber consists of non-starch polysaccharides such as arabinoxylans, cellulose, and many other plant components such as resistant starch, resistant dextrins, inulin, lignin, waxes, chitins, pectins, β-glucans, and oligosaccharides (Gaoshuang et al., 2012).

Thiamine is known to occur in the outer integuments and germ of cereal grain. In wheat grain, the endosperm represents 80–85% of grains dry mass (DM), but contains only 3% of the total thiamine. The highest proportion (80%) of thiamine is found in the external layers of wheat grain, but these are missing in white flour (Batifoulier et al., 2006). In the scientific literature are mentioned study reports on the thiamine and riboflavin contents of some Finnish cereal products including rye flour, crispbreads, and breakfast cereals, as well as their contents in some imported crispbreads and breakfast cereals sold in Finland. The mean thiamine contents of Finnish rye meal and flour (ash content 0.9–1.2%) were 0.28±0.01 and 0.27±0.04 mg 100 g⁻¹ fresh weight (FW), and their respective riboflavin contents were 0.10±0.01 and 0.08±0.02 mg 100 g⁻¹ FW. The thiamine contents of domestic crispbreads were 0.27±0.03 mg 100 g⁻¹ FW, and the riboflavin contents were 0.16±0.05 mg 100 g⁻¹ FW, which is significantly higher than levels in imported products. The thiamine contents of muesli and other breakfast cereals averaged 0.29±0.07 and 0.40±0.15 mg 100 g⁻¹ FW, respectively, and their riboflavin contents averaged 0.19±0.23 and 0.21±0.50 mg 100 g⁻¹ FW, respectively (Hägg et al., 1993).

Whole wheat flour, one of the most common and important whole grains, retains wheat bran and germ and acts as a rich source of dietary fibre, vitamins, minerals and antioxidants (Wang et al., 2014). Rye is second to wheat, the most commonly used grain in the food. Among the grain, rye is the only one with a wholegrain culture, and the consumption of rye should be increased in light of this benefit. In general, rye could be exploited more efficiently in new types of cereal products due to its positive health effects. Nowadays, its use is limited mainly as a result of the problems arising from its flavour; not all European consumers are familiar with the somewhat foreign, rye-like flavour, perceived as bitter and intense.
However, rye consumption in Europe might increase if ingredients were produced with the specific rye-like flavour modified to a slightly milder one, without significantly decreasing the contents of fibre and bioactive compounds in the rye. In general, volatile compounds that influence the cereal flavour have been extensively studied, but the sensory perception and, in particular, the methods of modifying the flavour of rye are not well known. With regard to the different flavour of the fractions of a rye grain, milling fractionation could be a valuable processing technique for modifying the rye-like flavour in the desired direction (Heiniö et al., 2003).

Triticale is a type of small grain created by genetically combining wheat and rye. Triticale grain, flours, and prepared products are available through both health food and commercial outlets on a limited basis. The data indicate that while the nutritional quality of triticale is considered superior to wheat, the higher ash content, lower milling yields of flour, and inferior loaf volume and texture distract from commercial baking use of triticale. In comparison with bread wheat, triticale has low gluten content, efficient gluten viscoelasticity and, therefore, inferior bread-making quality (Doxastakis et al., 2002).

Hull-less barley has been intensively investigated in respect to its food, feed and industrial applications. The advantage of hull-less barley compared to hulled barley in food uses is that pearlimg is not needed, so that the outer part of the endosperm, the aleurone, which contains proteins with essential amino acids and vitamins, is retained, as well as other bioactive compounds. It can be milled using conventional equipment available for wheat milling, with extraction yields of 73%. Hull-less barley flour has been successfully used in chemically leavened products such as muffins, pancakes, biscuits and cookies (Andersson et al., 2009a,b).

Traditionally, pasta is manufactured solely from durum wheat, which results in a product considered to be of superior quality to pasta made from cheaper common wheat or a blend of the two species. The manufacture of pastas from mixtures of durum and common wheat without adequate labelling is usually considered as adulteration. Durum wheat pasta for export outside the European Union may contain a maximum of 3% common wheat from unavoidable adventitious contamination during agricultural processing. Pasta is consumed in large quantities throughout the world. Scientific research has been undertaken to understand the parameters influencing pasta processing and the final product quality. Ideally, cooked pasta is of al dente quality. It is firm and resilient with no surface stickiness and little if any cooking losses (Sissons et al., 2005; Troccoli et al., 2000).

In the scientific literature practically was not found data about whole grain flour made from hull-less barley, rye, triticale and wheat application in pasta production, as well as dietary fibre and B₁ and B₂ vitamins content of possible whole flour blends for pasta making.

The main purpose of the current research was established to investigate total dietary fiber and vitamin B₁ and B₂ content in flour blends made from several types’ whole grain flour for pasta production.

Materials and Methods

The study was realised at the scientific laboratories of Faculty of Food Technology at Latvia University of Agriculture (LLU) and at the laboratory of the JSC Jelgavas dzirnavas (Latvia), at the laboratories of Latvia University Institute of Biology (Latvia).

Characterisation of raw materials

In 2012 harvested from State Priekuli Plant Breeding Institute (Latvia) convectional rye (‘Kaupo’), hull-less barley (line ‘PR 5099’) and triticale (line ‘9405-23’), as well as form LLU research station „Peterlauki” (Latvia) wheat (‘Zentos’) grain was used in the experiments. For the flour blend obtaining wheat flour 550 type (Latvia) was used.

Before experiments grain were grounded in laboratory mill Hawos (Hawos Kornmuhle GmbH, Germany) obtaining fine whole grain flour.

Preparation of flour blend samples

Whole grain blend proportions was pre-selected for the analysis of finished products – pasta. At the previous experiments optimal wheat flour 550 type and whole grain flours ratio was obtained as follow: 70% wheat flour 550 type and 30% whole wheat flour (W/W); 80% wheat flour 550 type and 20% whole rye flour (W/R); 70% wheat flour 550 type and 30% whole triticale flour (W/T); 80% wheat flour type and whole hull-less barley flour (W/H). As a control sample wheat flour 550 type was analysed.

Analysed parameters

Total dietary fiber content was measured by Fibertec system 1010 Heat Extractor corresponding to ISO 5498. Flour blend added 50 mL Phosphate buffer solution, pH 6.0, to each flask. The testing procedure was as follow: check pH and adjust if necessary to pH 6.0±0.2 by adding 0.275N NaOH or 0.325N HCl. Added 100 µL of Alpha amylase. Cover flasks with Aluminium foil and incubate in boiling water bath for 30 minutes. Adjust to pH 7.5±0.2 by adding 10 mL 0.275N NaOH than add 100 µL Protease solutions to each flask. Cover flasks with Aluminium foil and incubate in boiling water bath for 30 minutes at 60±1 °C. Dispense 10 mL 0.325N HCl into flasks while stirring. Adjust to pH 4.0-4.6. Add 200 µL Amyloglucosidase solutions while stirring. Cover flasks with Aluminium foil and incubate in boiling water bath for 30 minutes at 60±1 °C. To each digested sample added 280 mL 60 °C hot 95% Ethanol OH. Measure the volume of Ethanol OH prior to heating. Remove from bath and cover flasks with Aluminium foil. Let precipitate for 1h at room temperature. Wet and redistribute Celite bed in previously tarred crucible using a few mi 78% Ethanol OH from wash bottle.
Apply suction to draw Celite onto fritted glass as an even mat. Attach the crucibles and the Incubation flasks to the Fibertec E, Filtration Module according to instructions under section Filtration in AN 302. Filter alcohol-treated enzyme digested through crucible. Using wash bottle with 78% EtOH and rubber spatula transfer all remaining particles to crucible.

Vitamins B₁ and B₂ content was measured by AOAC Official methods 986.27 and 970.65 respectively.

**Mathematical data processing**

Data are expressed as mean ± standard deviation; for the mathematical data processing (p≤0.05) (ANOVA) was calculated.

**Results and Discussion**

The consumption of whole grains, which are rich in dietary fiber, resistant starch, vitamins, minerals and micro constituents, was reported to have many physiological benefits related to “western diseases” such as coronary heart disease, colon cancer and diabetes. However, the whole grain foods have not been attractive to consumers because the higher bran and germ in whole grain flour reduced the quality and sensory value of the end-use products (Hung et al., 2007).

**Dietary fiber**

Over the last decades, consumer demands in the field of industrially food production have changed considerably. For this reason, foods today are not intended only to satisfy hunger and to provide necessary nutrients, but also to prevent nutrition-related diseases and enhance physical and mental well-being of consumers. In this regard, functional foods offer an outstanding opportunity to improve the quality of products. Early functional foods were characterised by fortification with vitamins. Subsequently, the focus has shifted to foods enriched with dietary fibre (Foschia et al., 2013).

In the present research significant differences (p=0.007) in total dietary fiber content of analysed flour samples were established (Table 1).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Total dietary fiber, vitamin B₁ in B₂ content in flour samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flour sample</td>
<td>Dietary fiber, g 100g⁻¹</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Hull-less barley*</td>
<td>16.68±0.13</td>
</tr>
<tr>
<td>Rye*</td>
<td>16.71±0.12</td>
</tr>
<tr>
<td>Triticale*</td>
<td>12.80±0.14</td>
</tr>
<tr>
<td>Wheat*</td>
<td>11.66±0.11</td>
</tr>
<tr>
<td>Wheat 550 type</td>
<td>3.90±0.10</td>
</tr>
</tbody>
</table>

*whole grain flour

Higher dietary fiber content was found in whole grain hull-less barley and rye flour (Table 1); it was by approximately − 77% higher (p=0.008) comparing with wheat flour 550 type, by approximately − 23% higher (p=0.001) comparing with whole grain triticale flour and by approximately − 30% higher (p=0.001) comparing with whole grain wheat flour samples. However there are not found significant differences (p=0.445) in dietary fiber content in analysed whole grain hull-less barley and rye flour samples (Table 1).

According to the scientific literature, the total dietary fiber content of whole grain rye flour could be in the range from 18.7 to 22.2% (Andersson et al., 2009a), whole grain wheat flour – from 12.7 to 22.1% (Gebruers et al., 2008), whole grain triticale – from 13.2 to 16.0% (Rakha et al., 2011), hull-less barley – of 10.1 to 21.6% (Yalcin et al., 2007), what mainly corresponds to present results in this research.

As results of our experiments show, the lowest total dietary fiber content was obtained in wheat flour 550 type (Figure 1, Table 1), what mainly could be explained with only grain middle part milling. Lower analysed parameter content, as 14.786±0.148 g 100 g⁻¹ and 15.533±0.005 g 100 g⁻¹ (Figure 1), was obtained for whole wheat and triticale flour blend. However, the higher total dietary fiber content, as 19.833±0.175 g 100 g⁻¹ and 19.412±0.352 g 100 g⁻¹ (Figure 1) was obtained in whole rye and hull-less barley flour blend.

**Vitamins**

B vitamins all function as coenzymes in the central pathways by which fat and carbohydrate are metabolized within the cell. The vitamins are concentrated in the aleurone layer of cereal seeds and milling and refining of cereals can lead to large losses (Sanders et al., 2003).

According to the literature, in the present research higher B₁ and B₂ vitamin content was obtained in whole grain flour (Table 1) comparing with wheat flour 550 type, because of milling of grain all parts including aleurone layer.

Similar vitamin B₁ and B₂ content was found in whole grain rye and wheat flour samples. However lower vitamins content was found in wheat flour type 550.
During mathematical data processing it was detected, that B<sub>1</sub> vitamin content in whole grain rye and wheat flour was significantly (p=0.114) higher than in hull-less barley and triticale – by 25%; and by 46% higher (p=0.744) comparing with wheat flour type 550. Similar results were detected during analysing of vitamin B<sub>2</sub> content in flour samples. As a result lowest vitamin B<sub>2</sub> content was obtained in wheat flour type 550; it was by 52% lower comparing with vitamin content in whole grain rye and wheat flour, by 32% lower comparing with whole grain hull-less barley flour and by 37% lower comparing with whole grain triticale flour. Obtained results mainly could be explained with grain individuality and vitamin forming intensity during grain growing.

![Figure 2. Vitamins content in whole grain flour blends](image)

Lower B<sub>1</sub> vitamin content, as 2.7±0.14 mg kg<sup>-1</sup> was obtained for blend with whole hull-less barley flour (W/H). However, the higher, as 3.19±0.28 mg kg<sup>-1</sup>, was obtained for blend with whole wheat flour (W/W) (Figure 2). Differences mainly could be explained with grain individuality. In the scientific literature it is mentioned – grains are rich in thiamine, especially barley 0.356, mg 100 g<sup>-1</sup> (Lebiedzinska et al., 2006). Lower B<sub>2</sub> vitamin content was obtained for flour blend with whole hull-less barley flour (W/H), whole rye (W/R) and whole triticale flour (W/T) flour. However, the higher B<sub>2</sub> vitamin content, as 0.81±0.28 mg kg<sup>-1</sup> was obtained in flour blend with whole wheat flour (W/W) (Figure 2).

As a result it could be possible to increase the dietary fiber and B<sub>1</sub> and B<sub>2</sub> vitamins content in pasta by using of the whole-grain flour blends. Because in the scientific literature is mentioned, that, for example, wheat whole grain is a source of health-promoting phytochemicals in addition to traditional nutrients, including carbohydrates, proteins, lipids, vitamins, and minerals. Removal of wheat bran outer layers and wheat germ to produce semolina for pasta production significantly decreases phytochemicals and nutrients responsible for the health benefits of whole wheat grain. Whole wheat pasta is an ideal product for retention of the natural substances present in the durum wheat kernel. This chapter provides an overview of the relationship between whole grains and current health conditions, use of whole grain in pasta products, bioactive phytochemicals in whole grain and pasta, and, lastly, the major and minor nutrients in whole wheat pasta (Hirawan and Beta, 2014).

**Conclusions**

In the present research significant differences in total dietary fiber of analysed whole grain rye, hull-less barley, triticale and wheat flour samples were established. Higher dietary fiber content was found in whole grain hull-less barley and rye flour, lower – in whole wheat and triticale flour blend. B<sub>1</sub> and B<sub>2</sub> vitamin content in whole grain rye and wheat flour was significantly higher than in hull-less barley and triticale. Lower B<sub>1</sub> vitamin content, as 2.7±0.14 mg kg<sup>-1</sup> was obtained for hull-less barley flour blend, the higher as 3.19±0.28 mg kg<sup>-1</sup> for wheat flour blend. Lower B<sub>2</sub> vitamin content was obtained for flour blends made with whole hull-less barley, triticale and rye flour, however, the higher B<sub>2</sub>, as 0.81±0.28 mg kg<sup>-1</sup> in blend with whole wheat flour.

**Acknowledgment**

This research and publication has been prepared within the State Research Programme “Sustainable use of local resources (earth, food, and transport) – new products and technologies (NatRes)” Project No. 3. “Sustainable use of local agricultural resources for development of high nutritive value food products (Food)”.

**References**


NUTRITIONAL EVALUATION OF PULSE SPREADS IN COMPARISON TO NUTRIENT RECOMMENDATIONS
Asnate Kirse, Daina Karkliņa
Department of Food Technology, Faculty of Food Technology, Latvia University of Agriculture, Lieļa street 2, Jelgava, Latvia, e-mail: asnate.kirse@gmail.com

Abstract
Non-dairy and reduced fat/calorie spreads are becoming important for health conscious people; as well as increasing consumer choice, animal product alternatives have the potential to contribute to overall public health. Pulses and pulse products are primarily popular among vegetarian and health conscious consumers and could benefit people struggling with dietary changes. Therefore, the aim of this research was to analyse and collate nutritional values of four commercially available pulse (chickpeas or soy) spreads and one newly developed pulse (bean) spread in comparison to recommended daily allowance (RDA) of nutrients for adolescents and adults.

Bean spread was made of ground re-hydrated cooked seeds of beans, to which salt, spices and other ingredients were added. Macronutrients were determined according to standard methods. Nutritional values of commercially available pulse spreads were given according to product label information. The results show that there are significant nutritional differences among the tested pulse spreads (p<0.05). A serving (100 g) of pulse spreads covers 5.2–9.8% protein, 3.8–32.2% fat, 1.5–3.8% carbohydrates and 3.4–14.0% energy of RDA for adolescents while 6.9–12.0% protein, 5.7–46.8% fat, 1.7–4.1% carbohydrates and 4.2–16.8% energy of RDA is covered for adults.

A serving of pulse spreads covers the least of RDA for male adolescents and the most for female adults out of the four groups. Even though pulses are considered a good source of B vitamins, pulse spreads are low in all but folic acid. Solely the new bean spread also contains significant amount of thiamine.

Keywords: pulse spreads, nutritional value, recommended daily allowance.

Introduction
Non-dairy and reduced fat/calorie spreads are becoming important for health conscious people who are seeking attractive products from different sources. As well as increasing consumer choice, animal product alternatives have the potential to contribute to overall public health. Pulses can serve as the base raw materials with added advantages of lower price of products and increased protein content (Veena, Bhattacharya, 2012). Pulse products are primarily popular among vegetarian and health conscious consumers and could benefit people struggling with dietary changes.

Pulses (grain legumes) are dry seeds of leguminous plants which are distinguished from leguminous oil seeds (soy, peanuts) by their low fat content. Pulses also exclude such leguminous vegetables as green peas and snap beans which are immature legume pods and green seeds (Codex Alimentarius Standart 171-1989, Rev. 1, 1995). This must be taken into account since the best known legume spread is peanut butter; pulse seeds, however, are made from dry and then re-hydrated edible variety of bean, pea and lentil seeds.

Pulses (family Fabaceae) have been consumed for at least 10 000 years and are among the most extensively used foods in the world. Nutritionally, they are characterised by high protein content (about 20–30%), a very high proportion of carbohydrate (about 50–65%) and a very low fat content (about 1%). They are a significant source of many nutrients, including fibre, protein and iron, as well as B group vitamins (Mudryj et al., 2012).

Pulses contain a mixture of soluble and insoluble fibre, which lowers total serum and low-density lipoprotein (LDL) cholesterol and aids in gastrointestinal function, respectively (Tosh, Yada, 2010); pulse consumption results in higher intakes of fibre, carbohydrate, protein, Ca, K, folate, Zn, Fe and Mg, with lower intakes of saturated as well as total fat (Mitchell et al., 2009). Pulses are also gluten-free, so products made from pulse flours provide alternatives to wheat flour based products (Siddiq et al., 2013).

However, their consumption in the Western world remains quite low at less than 3.5 kg per capita per year. In other parts of the world, annual pulse consumption can range from 10 kg per capita (South America and India) to 40 kg per capita (Burundi) (Mudryj et al., 2012). Among European countries, higher pulse consumption is observed around the Mediterranean, with per capita daily consumption between 8 and 23 g, while in Northern Europe, the daily consumption is less than 5 g per capita (Bouchenak, Lamri-Senhadj, 2013). In 2009, legume (including pulses, soy and nuts) consumption represented only 0.7% of total regularly consumed foods in Latvian food basket, with daily consumption 32±2 g per capita (Joffe et al., 2009). According to the Latvian Central Statistical Bureau (CSB) data, the average pulse consumption was 9 g per capita per day in 2013, with annual consumption 3.28 kg per capita.

Commercially available pulse spreads are a fairly new concept, while the main spread-like vegetable protein product humus can be described as ancient food. Today, humus, a Middle Eastern and Arabic food dip or spread made from cooked, mashed chickpeas blended with tahini, olive oil, lemon juice, salt and garlic, is popular throughout the Middle East, North Africa, Mediterranean and in Middle Eastern cuisine around the globe (Marks, 2010).

There are about 10 different plant-derived spreads commercially available in Latvia varying a lot in...
nutritional value and ingredients. About half of them are oil, yeast or seed based and do not qualify as pulse (or legume) spreads. Latvian-produced spreads are more accessible to consumers in terms of price; however, they contain additives to ensure the texture or improve the structure of the product (thickeners, emulsifiers), improve the taste (flavour enhancing agents) and extend shelf life (preservatives).

The main difference in need for nutrients between adolescents and adults is higher total energy intake for adolescents because they experience greater increases in height, weight, and other aspects of body composition than adults; adolescent growth spurt is sensitive to energy and nutrient deprivation which can lead to delayed puberty or growth retardation (Stang Story, 2005). Adolescents also need higher intakes of healthy fat which is necessary for brain and nerve cell development and growth (Nettleton et al., 2013).

In order to determine the need for nutritionally improved products, one needs to ascertain the existing products on the market. Therefore, the aim of this research was to analyse and collate nutritional values of four commercially available pulse (chickpeas or soy) spreads and one newly developed pulse (bean) spread in comparison to recommended daily allowance (RDA) of nutrients for adolescents and adults.

Materials and Methods

Pulse (bean) spread with sun-dried tomatoes was prepared at the laboratory of Faculty of Food Technology (Latvia University of Agriculture) according to the vegetarian spread preparation technology in RL patent Vegetarian bean spread Technology (Latvia University of Agriculture). Beans or legumes, to which salt, spices and other ingredients were added (Kirse et al., 2013). Macro-nutrients were determined according to standard methods: protein content (AACC 46-20), fat content (AOAC 2003.06), total dietary fibre content (AOAC 994.13), ash content (ISO 2171:2010); available carbohydrates (g per 100 g of spreads) were determined by difference (Menezes et al., 2004) according to formula:

\[ \text{Available carbohydrates} = 100 - \left( \text{weight in grams} \left[ \frac{\text{protein} + \text{fat} + \text{water} + \text{ash}}{100 \text{ g of food}} \right] \right) \]

Nutritional values of commercially available pulse spreads are given according to product label information. Dietary fibre (for commercially available spreads) and vitamin content (mg or g per 100 g of spreads) was calculated according to formula:

\[ \text{Vitamin or dietary fibre} = n \times \frac{a}{b} \]

where \( n \) – the amount of nutrient (vitamin or dietary fibre) in 100 g of cooked pulses/legumes, \( a \) – protein content in the given spread (per 100 g), \( b \) – protein content in the cooked pulse/legume (per 100 g).

USDA National Nutrient Database for Standard Reference was used for dietary fibre and vitamin content reference in cooked pulses.

The calculation of the amount of nutrients needed (recommended daily intake of nutrients expressed as recommended daily allowance) for adolescents and adults has been done based on the ordinance No 174 Recommended allowance of energy and nutrients for Latvian citizens, issued by Ministry of Health of the Republic of Latvia on October 15, 2008. Calculations were carried out in both groups per gender (female and male) and average values were used for further analysis.

The obtained data processing was performed using mathematical and statistical methods with statistical software R 3.0.2; differences among results were considered significant if \( p \)-value \( <0.05 \). For the interpretation of the results it was assumed that \( \alpha=0.05 \) with 95% confidence (Næs et al, 2011). Differences among nutritional value of different pulse spreads were analysed using one way analysis of variance and Tukey’s test.

Results and Discussion

Five pulse spreads were compared in this study, four of which are commercially available: ILO Hummus by Silva, Finland (sample A), Hum-Hum Hummus by Zila Laguna, Latvia (sample B), WD fit soy spread by W-D, Poland (sample C), BioGreno Curry-Pineapple spread by Bartels-Langness, Germany (sample D) and bean spread with sun-dried tomatoes experimentally developed at the laboratory of Faculty of Food Technology in Latvia University of Agriculture, Latvia (sample E). General information about the investigated spreads is given in Table 1.

<table>
<thead>
<tr>
<th>Pulse spreads</th>
<th>Type of pulses used</th>
<th>Approximate shelf life</th>
<th>Price, EUR kg -1</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Chickpeas <em>Cicer arietinum</em> L.</td>
<td>8 weeks*</td>
<td>14.70</td>
</tr>
<tr>
<td>B</td>
<td>Chickpeas</td>
<td>4 weeks*</td>
<td>6.20</td>
</tr>
<tr>
<td>C</td>
<td>Soy <em>Glycine max</em> (L. Merr.)</td>
<td>4 weeks</td>
<td>8.00</td>
</tr>
<tr>
<td>D</td>
<td>Chickpeas and soy</td>
<td>8 months</td>
<td>13.80</td>
</tr>
<tr>
<td>E</td>
<td>Beans <em>Phaseolus vulgaris</em> L.</td>
<td>7 days</td>
<td>5.60**</td>
</tr>
</tbody>
</table>

* preservatives used
** price forecast

Data presented in Table 1 show that chickpeas and soy are the main legumes used in commercially available non-dairy spreads. As mentioned before chickpeas and soy are both legumes but belong to different subtypes of legumes, pulses and leguminous oil seeds, respectively. In this study soy spread has been included because soy is used for the content of protein not oil.

There are significant differences among the shelf life of these spreads, e.g. sample D has the longest shelf life
because curry-pineapple spread is filled in small glass bottles and heat treatment (pasteurisation) has been performed. Spreads A and B (hummus) have the shelf life up to two months because preservatives (sorbic acid E200, lactic acid E270, potassium sorbate E202) have been used. The shelf life of bean spread with sun-dried tomatoes is only one week because no heat treatment or preservatives were added and the proper packaging is currently being researched. The shelf life of bean spread with sun-dried tomatoes is equal to the expiration date of commercially available spreads when kept in the refrigerator after opened.

There are also significant differences among the price of the investigated spreads. The price of three spreads (B, C and E) is less than 10 EUR kg⁻¹ and it is comparable to the price of dairy spreads. The ingredients of spread D have been grown in biological agriculture and this could be the reason for a higher price. The price of spread A could be higher because of increased marketing margin and raw materials of higher quality and price.

Nutritional value of pulse spreads is given in Table 2. Nutritional information of samples A-D is given according to product label information: protein, fat and carbohydrate content in bean spread with sun-dried tomatoes has been determined experimentally according to standard methods.

Table 2

<table>
<thead>
<tr>
<th>Pulse spreads</th>
<th>Protein, g</th>
<th>Fat, g</th>
<th>Carbohydrates, g</th>
<th>Energy, kcal</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6.0⁺</td>
<td>10.0⁺</td>
<td>8.0⁺</td>
<td>146.0⁺</td>
</tr>
<tr>
<td>B</td>
<td>6.5⁺</td>
<td>29.0⁺</td>
<td>12.0⁺</td>
<td>335.0⁺</td>
</tr>
<tr>
<td>C</td>
<td>6.0⁺</td>
<td>22.2⁺</td>
<td>6.0⁺</td>
<td>247.8⁺</td>
</tr>
<tr>
<td>D</td>
<td>5.2⁺</td>
<td>4.3⁺</td>
<td>10.6⁺</td>
<td>101.9⁺</td>
</tr>
<tr>
<td>E</td>
<td>7.8⁺</td>
<td>8.3⁺</td>
<td>8.4⁺</td>
<td>139.5⁺</td>
</tr>
</tbody>
</table>

* values within a column not sharing a superscript letter are significantly different (p<0.05)

Spread E has the highest protein content out of all investigated spread samples (p=0.012). Commercially available non-dairy spreads contain significantly less protein and spread D has the least protein. Literature data confirm that white beans can contain significantly more protein than chickpeas and soy (Maskus, 2010). Fat content in pulse spreads is significantly higher comparing with dry and cooked pulses (p<0.001) because of the oil added for a better texture. Spreads A, D and E have the least fat and they qualify as nondairy, reduced fat spreads. Hummus produced in Latvia (spread B) already has the highest fat content, nevertheless, it is recommended to add more oil before eating it according to product label information.

Carbohydrates available for digestion by human enzymes account to less than 15 g 100 g⁻¹ of pulse spreads; even though there are significant differences among five spread samples, pulse spreads are low in carbohydrates.

Bean spread with sun-dried tomatoes (spread E) contains 9.83±0.10 g 100 g⁻¹ total dietary fibre; commercially available non-dairy spreads are not labelled as containing fibre. According to Commission Directive 2008/100/EC and Regulation No 1924/2006, products can be labelled as a source of protein if at least 12% of the energy value of the food is provided by protein. Spreads A, D and E are a source of protein because 16%, 20% and 22% of the energy value is provided by protein, respectively. The same regulations allow labelling bean spread with sun-dried tomatoes (spread E) as high in fibre considering it contains ≥6.0 g fibre 100 g⁻¹.

A claim that a food is energy-reduced may be made if the energy value of the product is reduced by at least 30% with an indication of the characteristic(s) which make(s) the food reduced in its total energy value. Fat content is the characteristic which makes three spreads (A, D and E) energy-reduced as energy value of those spreads is 40.8% to 69.5% lower compared to spreads B and C.

The calculation of the amount of nutrients needed for adolescents and adults has been done based on the ordinance No 174 Recommended allowance of energy and nutrients for Latvian citizens, issued by Ministry of Health of the Republic of Latvia on October 15, 2008. Reference values of recommended daily allowance (RDA) of nutrients are given in Table 3. The data have been used in subsequent calculations.

Table 3

<table>
<thead>
<tr>
<th>Age group</th>
<th>Energy (E), kcal</th>
<th>Protein, E%</th>
<th>Fat, E%</th>
<th>Carbohydrates, E%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adolescent</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>– male</td>
<td>3000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>– male</td>
<td>2400</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*average values were used for further calculations

Recommended daily energy intake for adolescent females and adult males is 10 042 kJ per day, while the amount is lower for adult females and higher for adolescent males, 8 368 kJ per day and 12 552 kJ per day, respectively. Adolescent males have higher caloric requirements since they experience greater increases in height, weight, and lean body mass than females. Protein needs for adolescents and adults are similar. (Stang, Story, 2005)

Recommended daily intake for fat of total energy intake is higher for adolescents. The human body requires dietary fat and essential fatty acids for normal growth and development; healthy fat (composed of mono- and polyunsaturated fatty acids) is necessary for brain and nerve cell development and growth, which is especially important for growing teens. Fat-soluble
vitamins A, D, E and K cannot function without adequate daily fat intake. (Nettleton et. al., 2013)

The calculation of the amount of nutrients needed for adolescents and adults was completed as the coverage of a nutrient by one serving (100 g) of pulse spreads in comparison to RDA.

The recommended daily intake of protein is 80 g for female adolescents, 100 g for male adolescents, 65 g for adult females and 75 g for adult males. A serving of pulse spreads covers 5.2–9.8% protein for adolescents and 6.9–12.0% protein for adults (Fig. 1).

![Figure 1. Protein coverage (%) of one serving of pulse spreads in comparison to RDA](image)

As spread E has the highest protein content, protein coverage of bean spread with sun-dried tomatoes is the best. Spread E can be considered a good source of protein for adolescents. Protein coverage for adolescent males is the lowest because the requirements for protein in this group are the highest.

The recommended daily intake of fat is 90 g for female adolescents, 113 g for male adolescents, 62 g for adult females and 75 g for adult males. A serving of pulse spreads covers 3.8–32.2% fat for adolescents and 5.7–46.8% fat for adults (Fig. 2). There are significant differences among the coverage of fat in the four groups (p<0.05).

![Figure 2. Fat coverage (%) of one serving of pulse spreads in comparison to RDA](image)

Spreads B and C cover the most of fat compared to the other three spreads. Hummus produced in Latvia (spread B) covers almost half of recommended daily intake for adult females. Contrary to protein, fat coverage as high from one serving cannot be considered a good indicator. A serving of spreads B or C cannot be recommended for daily consumption. Fat coverage for adolescent males is the lowest once more because the requirements for fat in this group are the highest.

The recommended daily intake of carbohydrates is 320 g for female adolescents, 400 g for male adolescents, 295 g for adult females and 355 g for adult males. A serving of pulse spreads covers 1.5–3.8% carbohydrates for adolescents and 1.7–4.1% carbohydrates for adults (Fig. 3).

![Figure 3. Carbohydrate coverage (%) of one serving of pulse spreads in comparison to RDA](image)

Compared to RDA, carbohydrate coverage is low for all investigated pulse spreads. As mentioned before, commercially available non-dairy spreads are not labelled as containing fibre, however, after calculations it can be concluded that dietary fibre content in spreads A-C is around 5.2 g 100 g⁻¹ and spread D contains at least 4.0 g 100 g⁻¹ dietary fibre. Bean spread with sun-dried tomatoes (spread E) contains more total dietary fibre (9.83±0.10 g 100 g⁻¹) than other pulse spreads (p=0.009).

According to European Guidelines on cardiovascular disease prevention recommended dietary fibre intake is 30–45 g per day or 3.4 g per 1000 kJ (Perk et al., 2012). While Guideline Daily Amounts (GDA) Labelling Initiative (by The Confederation of the Food and Drink Industries of the EU) recommends 25 g
dietary fibre per day for both genders (reference amount for 2000 kcal diet) but no further recommendations for diets in lower or higher calories are given. Therefore, if European Guidelines on cardiovascular disease prevention are used as reference for optimal dietary fibre intake, a serving of pulse spreads covers 12–21% fibre for adolescent males, 15–26% fibre for adolescent females and adult males and 18–31% fibre for adult females. A serving of pulse spreads covers 3.4–14.0% energy for adolescents and 4.2–16.8% energy for adults (Fig. 4).

Spreads B and C cover as much as twice the amount of calories per one serving of other pulse spreads; most of the calories come from fat. As mentioned before spreads A, D and E are energy-reduced and can be considered a good source of macronutrients for daily consumption.

Pulses are a good source of B vitamins, namely B1, B3, niacin, pyridoxine and folic acid (Ofuya et al., 2005); however, pulse spreads are low in most of them (Fig. 5). According to Regulation No 1924/2006 a claim that a food is a source of vitamins can be made if the product contains at least a significant amount, i.e., 15% of the recommended daily allowance (which in absolute numbers is equal for adolescents and adults). All pulse spreads except spread D are a source of folic acid because soy has lower amounts of the vitamin previously classified as B9. Solely bean spread with sun-dried tomatoes also contains a significant amount of thiamine (p=0.008).

**Conclusions**

A serving of pulse spreads (including soy spread) covers the least of RDA for male adolescents and the most for female adults out of the four groups. Even though pulses are considered a good source of B vitamins, pulse spreads are low in all but folic acid. Solely the new bean spread also contains a significant amount of thiamine.

Nutritional value of pulse spreads is essentially different, depending on the raw materials used for spread production.

**References**


EXTRUDED BEAN PRODUCT QUALITY EVALUATION

Liene Strauta1, Sandra Muizniec-Brasava1, Ina Alsina2, Tatjana Rakcejeva3
1 Faculty of Food Technology, Latvia University of Agriculture, Liela street 2, Jelgava, Latvia, e mail: strauta.liene@gmail.com
2 Faculty of Agriculture, Latvia University of Agriculture, Liela street 2, Jelgava, Latvia

Abstract
Aim of study was to develop a new type of bean (Phaseolus vulgaris L.) products with various additives using one screw extrusion, define their quality (physical and chemical properties) and ascertain people opinion about such products. Research was carried out at Latvia University of Agriculture Faculty of Food Technology. White beans were used in experiments. Beans were boiled, crushed and extruded through one screw extruder. Different additives were used, as bell peppers, tomatoes, spinach, garlic and red beet. Parameters as crude protein content, ash content, pH and colour were determinate. People at international exhibition “Riga Food 2013” were asked to give their opinion about the experimental product.
Average protein content in beans (Phaseolus vulgaris L.) extruded products were 25.5±0.4% of dry matter (DM). Average ash content was 4.99±0.05% of DM and pH 5.77. Dry matter was 91.59±0.03% in average. No more than 5% of lignified protein had been found in product.

Keywords: beans, extrusion, protein, ash.

Introduction
Dietary importance of legumes has been well established. Their high content of protein, carbohydrates, fiber, certain minerals and vitamins make them a good source of nutrients. Nowadays research on nutraceutical properties of beans is a very hot topic (Rocha-Guzma et al., 2006).

Kidney bean (Phaseolus vulgaris L.) is the most widely produced and consumed food legume not only in Africa, India, Latin America and Mexico (FAO, 1993) but worldwide. These beans have reported to have numerous health benefits, e.g. heart and renal disease risks (Anderson et al., 1999); lower glycemic index for persons with diabetes (Viswanathan et al., 1989); increased satiation (Leathwood, Pollet, 1988) and cancer prevention (Hangen, Bennink, 2002). Furthermore, kidney beans are regarded as an important source of protein and minerals for livestock feed production, as well as, potential raw materials for processing into human food (Gupta, 1987; Salunkhe, 1982; Kahlon et al., 2005).

The low incidence of diseases related to blood circulation in Asian population is ascribed, in part, to a relatively high consumption of legumes (Kahlon et al., 2005).

However, legumes are an underutilised commodity in most regions of the world due to long cooking time (Deshpande et al., 1984; Nasar-Abbas et al., 2008), and the belief that legumes are of low nutritive value due to the presence of flatulence and anti-nutritional factors (Adsule et al., 1989). However, legumes are potentially valuable dietary components due to a protein content between 20% and 50%, and the presence of complex carbohydrates, especially dietary fibre and water soluble polysaccharides, which give them low glycaemic potency (Ravindran et al., 2011).

The antinutrients (e.g. trypsin inhibitors, phytic acid, saponins, phytohemagglutinins and tannins) and α-galactosides (e.g. raffinose, stachyose and verbascose) are some of the undesirable components in beans that could limit their protein and carbohydrate utilization (Shimelis, Rakshit, 2007).

Unfortunately, the antinutritional factors reduce biological activity of several chemical compounds or metabolites (Rocha-Guzma et al., 2006).

All processing methods tend to modify the composition and availability of nutrients in raw materials. Among the various technologies, short-time high-temperature extrusion cooking is a well known cost-effective industrial process. Extrusion combines high pressure with a moderately high-temperature and usually high shear for a short period of time. Extrusion processing completely gelatinises the starch and partially or completely destroys antinutritional factors present in many legumes (Melcion, Poel, 1993; Ravindran et al., 2011). Extrusion-cooking technology is a versatile and efficient method of converting raw materials into finished food products. It can replace many conventional processes in food and feed industries. It has been used to develop various types of snack foods, mainly from corn meal, rice, wheat flour, or potato flour, in many shapes and variety of textures. Application of extrusion process for legume flours is a relatively new area, with the exception of soy bean. Several reports show that bean starches have very good expansive and functional properties under extrusion conditions (Gujka, Khan, 1991). According to Gujka, Czarnecki, and Khan (1996), extrusion cooking has good potential for making desirable bean forms that could be economically available in developing countries. Benefits of beans extrusion-cooking are deactivation of heat labile growth inhibitors (Aguilera et al., 1984). Sosulski (1988) claims that legumes flour has not been used widely in foods because of the poor functionality of starch (Rocha-Guzma et al., 2006).

Aim of this research was to develop a new type of bean (Phaseolus vulgaris L.) products with various additives using one screw extrusion, define their quality (physical and chemical properties) and ascertain people opinion about such products.

Materials and Methods
White beans (Phaseolus vulgaris L.) were boiled, then crushed with hand meat-grinder and extruded by
Crude protein (CP) was determined using Kjeldahl method. Approximately 0.5 g (to the nearest 0.0001 g) of well crushed sample was weighed. The sample was transferred in a temperature resistant glass flask; where copper catalyst was added and 20 mL of concentrated H_2SO_4. Flask was placed in a preheated BUCHI digest automat K-438 (420±5 °C) and gas vacuum suction cap was fixed on. Wet mineralization went for 1 hour until the solution in flask was colourless and clear. Flask and digested sample was removed from the stove and allowed to cool for 15–20 min. Cooled sample was placed in a distillation unit, 50 mL of water, 80 mL of 40% NaOH were added. Ammonium was distilled in 65 mL of 4% H_2BO_3 solution. The steam distillation was carried out for 220 s. Boric acid solution was titrated with 0.2 M HCl (which concentration was checked with 0.1 M NaOH solution) till pH 4.70. 1 g of sucrose was used as a blank sample. For protein calculations coefficient 6.25 was used (LVS EN ISO 5983-2, 2009).

Ash content was determined according ISO 5984:2002/Cor 1:2005. The sample mass 5±0.0001 g was weighed, placed in the porcelain crucible and placed on Bomanstove. After 20 min sample was placed in muffle for 4 hours at 550 °C temperature. When muffle has cooled till 100 °C temperature samples are placed in an exsiccator and cooled till room temperature, then weighted on analytic scale.

Lignified protein content was determinate in two steps. First, acid detergent fibre content was determined according to LVS EN ISO 13906, 2008. Borosilicate crucible is dried for 4 hours at 103±3 °C, and weighted to the nearest 0.1 mg. Sample is taken 1000±2 mg. Cruicible is placed in the analyzer. 100 mL of the ADF solution is added and cooling water is switched on after 5–10 min. After 60±5 min the hardware glass walls are washed with less than 5 mL of ADF solution. The heater was turned off and solution filtered off. The sample was washed 3 times with 30 mL of hot (90–100 °C), distilled water allowing it to stay on sample for 3–5 min. After washing crucible with the sample was transferred to the cold extraction unit and washed with 25 mL of acetone and filtered. Then crucible with the sample was placed in the drying oven at 103±3 °C for more than 5 hours or 130±3 °C for more than 2 hours (LVS EN ISO 13906, 2008).

Second, after determining the ADF fraction, dried sample was transferred in high temperature resistant glass flask and proceeded the same as crude protein. For lignified protein determination sample weigh before ADF determination is used in calculations (Undersander et al., 1993).

Sodium and iron was determined according to LVS EN ISO 6869. After ash content was determined, ash was transferred in to volumetric flask and added 10 mL 6 M HCl solution. Solution was dried on hot plate. Then residue dissolved in 5 mL 6 M HCl and transferred with some 5 mL portions of hot water in 50 mL volumetric flask. Cooled and diluted to the mark with

L Series Göttfert Werkstoff Single screw laboratory extruder Different additives were added before extrusion (Table 1). Additives used in experiment were as shown in Table 1.

### Table 1

<table>
<thead>
<tr>
<th>No</th>
<th>Additive</th>
<th>Amount of additive, %</th>
<th>Amount of salt, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (without additive)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Chia seeds</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>Nettle powder</td>
<td>3</td>
<td>0.3</td>
</tr>
<tr>
<td>4</td>
<td>Caraway-seeds</td>
<td>1</td>
<td>0.3</td>
</tr>
<tr>
<td>5</td>
<td>Fresh bell pepper</td>
<td>20</td>
<td>0.5</td>
</tr>
<tr>
<td>6</td>
<td>Salt</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>Beetroot</td>
<td>12</td>
<td>0.3</td>
</tr>
<tr>
<td>8</td>
<td>Spinach</td>
<td>12</td>
<td>0.3</td>
</tr>
<tr>
<td>9</td>
<td>Almond essence</td>
<td>0.5</td>
<td>0.1</td>
</tr>
<tr>
<td>10</td>
<td>Lemon essence</td>
<td>0.5</td>
<td>0.1</td>
</tr>
<tr>
<td>11</td>
<td>Thyme powder</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>12</td>
<td>Tomato paste</td>
<td>15</td>
<td>0.2</td>
</tr>
<tr>
<td>13</td>
<td>Garlic powder</td>
<td>1</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Sample without additives was used as a control. Extrusion process was performed at temperature regimes 90–95–105 °C. Before extrusion dry matter of samples was measured using dry matter weights Precisa XM120.

After extrusion samples (size of one piece – 15×20 mm) were dried in a rotary type oven SVEBA DAHLIN S400 for 2 hours at 90 °C temperature. Dry samples were packaged in conventional two layer laminated polymer polyethylene/polyamide (PE/PA) pouches (200×300 mm, material thickness 20/45±2 µm) and then hermetically sealed by chamber type machine MULTIVAC C300. Mass of the sample in each package was 100±5 g. Quality of the extruded products was analysed at Faculty of Food Technology and Scientific laboratory of Agronomy Research. Some of samples were tested at the international exhibition “Riga Food 2014”, to roughly estimate the consumers’ interest in such products.

The total dry matter content was determined according to ISO 6496, 1999. Container with lid for half an hour was dried at 103±3 °C temperature, then cooled to room temperature in desiccators. Weigh was measured with an accuracy of 1 mg. 5 g of sample weight of to the nearest 1 mg were placed in container and put in the drying oven at 103±3 °C for 4.0±0.1 h. After four hours, the container lid were put on, sample was removed from oven and cooled in a desiccators to room temperature. Sample was weighted with container and absolute dry matter was calculated according to method. All chemical parameters were calculated on absolute dry matter.
water. After particles had settled, a dilution factor of water was added, 100 mL of lanthanum nitrate solution, 5 mL of caesium chloride solution and 5 mL of 6 M HCl acid solution. Then atomic absorbance is measured. Solution is diluted as much is needed to obtain absorbance in the linear part of calibration curve, which is made from known sodium or iron quantity solution. Blank sample is made using water instead of the test sample. pH was determined according to ГОСТ 26180-84. 10 g of well-crushed sample was placed in a volumetric flask. Water was added till 100 mL. After 5 hours pH was measured. Colour of product was measured in CIE L*a*b* colour system using Tristimulus Colorimeter, measuring Hunter colour parameters by Colour Tec PCM/PSM. Colour values were recorded as L* (brightness) – the vertical co-ordinate runs from L* = 100 (white) through grey to L* = 0 (black); a* (-a, greenness, +a, redness) – the horizontal co-ordinate, that runs from -a* (green) through grey to +a* (red) and b* (-b, blueness, +b, yellowness) – another horizontal co-ordinate, that runs from -b* (blue) through grey to +b* (yellow) (Papadakis et al., 2000). Samples were crushed and bolted through 1 mm sieve. Samples were densely pressed in a Petri plate with diameter 5 cm and colour was measured at least ten times at randomly selected locations of each sample. Colour difference (ΔE*) of bean products from control sample was calculated using the following equation 1:

\[ \Delta E^* = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \]  

(1)

where ΔE* – colour difference of the product, L* – products colour intensity value, L_0* – control samples colour intensity value, a* – products value of colour component green – red, a_0* – control samples value of colour component green – red, b* – products value of colour component blue – yellow, b_0* – control samples value of colour component blue – yellow.

During international exhibition “Riga Food 2013” 150 exhibition visitor as potential consumer opinion about such products were asked using 5 point scale for likeness. Where score 1 means “does not like at all” and score 5 – “likes very much”. Data were analysed with correlation, ANOVA single factor analyses using Microsoft Excel 2007. Data Analysis, confidence level was taken 95% (α=0.05).

**Results and Discussion**

The dry matter content of prepared samples before extrusion was 38.0 g 100 g\(^{-1}\) in average (Figure 1). Sample with bell peppers contained only 34.6±0.5 % of dry matter, while samples with chia seeds and nettles– 38.8±0.5 % of dry matter each. The largest dry matter 39.30±0.5 % was detected in a sample with spinach additive.

![Figure 1. Dry matter of samples before extrusion](image)

1 – control 2 – chia seeds; 3 – nettle; 4 – caraway seeds; 5 – bell peppers; 6 – salt; 7 – beetroot; 8 – spinach; 9 – almond essence; 10 – lemon essence; 11 – thyme; 12 – tomato; 13 – garlic; – line for control sample

Dry matter in extruded bean products is shown in Figure 2. The highest dry matter content was in samples with bell peppers, 93.13±0.01 g 100 g\(^{-1}\), and samples with salt 93.07±0.03 g 100 g\(^{-1}\) but the lowest in a sample with almond essence – 88.45±0.02 g 100 g\(^{-1}\), and in control sample it was 95.13±0.05 g 100 g\(^{-1}\) of product, that could be explained with vaporization of essence.

![Figure 2. Dry matter in extruded bean products](image)

1 – control 2 – chia seeds; 3 – nettle; 4 – caraway seeds; 5 – bell peppers; 6 – salt; 7 – beetroot; 8 – spinach; 9 – almond essence; 10 – lemon essence; 11 – thyme; 12 – tomato; 13 – garlic; – line for control sample

Significant differences were found in protein content (Figure 3) among extruded products (p=3.2×10\(^{-5}\)). The highest protein content was found in garlic containing products – 26.7±0.3 g 100 g\(^{-1}\) of product, but the less in those ones with tomato – 23.2±0.2 g 100 g\(^{-1}\) of product. They were the ones that made the changes significant. Mathematical analysis showed that there was no significant difference among other investigated sample protein content on dry matter. Protein content in a control sample was 26.2±0.2 g 100 g\(^{-1}\). Relatively high protein content of samples with spinach can be explained b fact that fresh, raw spinach contains till 30% protein of dry matter. So the crude protein loss was not significant despite fresh material.
The content of ash in extruded bean products is shown Figure 4. The lowest ash content is determined in control sample – 3.46±0.07 g 100 g⁻¹ of products dry matter, sample with lemon essence – 3.49±0.04 g 100 g⁻¹ of products dry matter and almond essence – 3.52±0.01 g 100 g⁻¹ of products dry matter.

However the highest ash content was detected in samples with salt and chia seeds addition 7.98±0.01 g 100 g⁻¹, but with chia seeds – 7.97±0.09 g 100 g⁻¹, as it is indicated in feed database (Interactive, 2014), so we can conclude, that no significant amount of lignified protein of CP can be even 8.2 g 100 g⁻¹ of product. Thus we can see control sample contains only little traces of sodium.

Lignified protein content of crude protein (CP) in samples was 3.40±0.04 g 100 g⁻¹, however in beans lignified protein of CP can be even 8.2 g 100 g⁻¹, as it is indicated in feed database (Interactive, 2014), so we can conclude, that no significant amount of lignified protein has been formed in extrusion process.

Sodium content in control sample was 0.0253±0.0002 g 100 g⁻¹, but with chia seeds – 1.93±0.03 g 100 g⁻¹ and in the sample with salt 2.09±0.07 g 100 g⁻¹ of product. Thus we can see control sample contains only little traces of sodium.

Control sample and sample with beetroot had no significant differences. Control sample contained 90±2 mg kg⁻¹ iron in dry matter of and sample with beetroot contained 91.4±0.3 mg kg⁻¹ iron in dry matter. Samples with chia seeds and spinach had higher iron content 111±2 mg kg⁻¹ and 103±1 mg kg⁻¹, respectively.

The values of extruded bean sample colour components L*, a* and b* are summarized in Table 2.

Table 2

<table>
<thead>
<tr>
<th>Added additive</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (without additive)</td>
<td>82.9±0.3</td>
<td>-1.5±0.1</td>
<td>15.9±0.3</td>
</tr>
<tr>
<td>Chia seeds</td>
<td>78.6±0.2</td>
<td>-1.1±0.3</td>
<td>14.3±0.4</td>
</tr>
<tr>
<td>Nettle</td>
<td>63.2±0.4</td>
<td>-1.7±0.1</td>
<td>16.6±0.4</td>
</tr>
<tr>
<td>Caraway-seeds</td>
<td>75.4±0.7</td>
<td>-1.5±0.3</td>
<td>17.5±0.4</td>
</tr>
<tr>
<td>Bell peppers</td>
<td>71.8±0.4</td>
<td>2.6±0.3</td>
<td>24.7±0.6</td>
</tr>
<tr>
<td>Salt</td>
<td>76.4±0.6</td>
<td>-1.1±0.3</td>
<td>15.5±0.8</td>
</tr>
<tr>
<td>Beetroot</td>
<td>55.9±0.5</td>
<td>16.8±0.4</td>
<td>1.1±0.2</td>
</tr>
<tr>
<td>Spinach</td>
<td>69.7±0.3</td>
<td>-2.0±0.2</td>
<td>19.9±0.4</td>
</tr>
<tr>
<td>Almond essence</td>
<td>77.8±0.3</td>
<td>-1.4±0.1</td>
<td>17.2±0.3</td>
</tr>
<tr>
<td>Lemon essence</td>
<td>78.0±0.2</td>
<td>-1.7±0.2</td>
<td>16.4±0.6</td>
</tr>
<tr>
<td>Thyme</td>
<td>74.7±0.2</td>
<td>-1.5±0.3</td>
<td>15.5±0.4</td>
</tr>
<tr>
<td>Tomato</td>
<td>74.7±0.3</td>
<td>-0.8±0.2</td>
<td>22.2±0.3</td>
</tr>
<tr>
<td>Garlic</td>
<td>76.0±0.4</td>
<td>-1.3±0.2</td>
<td>16.3±0.5</td>
</tr>
</tbody>
</table>

Sample with added beetroot was darker, described by the lowest L* value 55.9±0.5 as well as the highest red one – 16.8±0.4 and the smallest b* value 1.1±0.2. While the lightest one was found control sample without additives described by colour components as follows: L*=82.9±0.3; a*=-1.5±0.1 and b*=15.9±0.3. The changes of colour components compared to a control sample are summarized in Table 3.

The colour of sample with beetroot differs from other extruded products.
The changes of colour components compared to control sample

<table>
<thead>
<tr>
<th>Added additive</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>∆E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chia seeds</td>
<td>-4.3</td>
<td>0.4</td>
<td>-1.6</td>
<td>4.6</td>
</tr>
<tr>
<td>Nettle</td>
<td>-19.7</td>
<td>-0.2</td>
<td>0.7</td>
<td>19.7</td>
</tr>
<tr>
<td>Caraway-seeds</td>
<td>-7.5</td>
<td>0.0</td>
<td>1.6</td>
<td>7.7</td>
</tr>
<tr>
<td>Bell peppers</td>
<td>-11.1</td>
<td>4.1</td>
<td>8.8</td>
<td>14.7</td>
</tr>
<tr>
<td>Salt</td>
<td>-6.4</td>
<td>0.4</td>
<td>-0.4</td>
<td>6.5</td>
</tr>
<tr>
<td>Beetroot</td>
<td>-27.0</td>
<td>18.3</td>
<td>-14.8</td>
<td>35.8</td>
</tr>
<tr>
<td>Spinach</td>
<td>-13.2</td>
<td>-1.2</td>
<td>3.9</td>
<td>13.8</td>
</tr>
<tr>
<td>Almond essence</td>
<td>-5.1</td>
<td>0.1</td>
<td>1.3</td>
<td>5.3</td>
</tr>
<tr>
<td>Lemon essence</td>
<td>-4.9</td>
<td>-0.2</td>
<td>0.5</td>
<td>4.9</td>
</tr>
<tr>
<td>Thyme</td>
<td>-8.2</td>
<td>0.0</td>
<td>-0.4</td>
<td>8.2</td>
</tr>
<tr>
<td>Tomato</td>
<td>-8.2</td>
<td>0.7</td>
<td>6.2</td>
<td>10.3</td>
</tr>
<tr>
<td>Garlic</td>
<td>-6.9</td>
<td>0.2</td>
<td>0.4</td>
<td>6.9</td>
</tr>
</tbody>
</table>

Correlation was observed between Na and Fe content (r=0.98); negative correlation between Na and pH (r=0.96); dry matter and Fe content (r=0.88); Na and dry matter content (r=0.86); negative correlation between protein and Na – r=-0.68 and protein and pH content (r=0.64).

The visitors in International exhibition “Riga Food 2014” were surveyed for their opinion of extruded bean products. Most likable to respondents seemed product with bell peppers and salt (3.6 and 3.3 points on average in 5 point scale), but the least they liked products with caraway seeds and lemon essence (2.6 and 2.8 in 5 point scale). Other tasted products were valued approximately by 3 points in 5 point scale.

Conclusions

Dry matter content of extruded bean products was in range from 88.45 g 100 g⁻¹ to 93.13 g 100 g⁻¹, in a control sample it was 92.15 g 100 g⁻¹. Protein content was in range 23.49 g 100 g⁻¹ till 26.69 g 100 g⁻¹, in control sample – 26.21 g 100 g⁻¹, and ash content 3.46 till 7.98 g 100 g⁻¹. Highest iron content was in samples with chia seeds 111±2 mg kg⁻¹ on dry matter and spinach and 103±1 mg kg⁻¹ on dry matter.

pH value was in range from 5.26 to 6.35. The highest pH has a control sample, all samples with additives had lower pH, except for lemon and almond essences. As expected high correlation have been observed between ash and sodium and iron content (r=0.98) and sodium and pH (r=-0.96). Not so close correlation was observed between dry matter and iron content (r=0.88), protein and sodium content (r=-0.68), protein and pH content (r=0.64) and sodium and dry matter content before extrusion (r=0.86).

References


So we can see that beetroot gives samples the darkest colour, followed by nettle and spinach. Red colour is more in samples with bell peppers too, but not as much as in one with beetroot. Green colour can be obtained with nettle, spinach and lemon essence additives but yellow with bell peppers, tomato and spinach additives.

pH was measured and significant differences were found (p=1.15×10⁻³⁰). All samples can be divided in four groups, according to pH (Fig. 6).

Figure 6. pH of extruded bean products

1 – control 2 – chia seeds; 3 –nettle; 4 –caraway seeds; 5 – bell peppers; 6 – salt; 7 – beetroot; 8 – spinach; 9 – almond essence; 10 – lemon essence; 11 – thyme; 12 – tomato; 13 – garlic

Products with pH=6.0 relates to the first group including control sample as well as sample with spinach additive, the second one with pH 5.8–6.0 would contain samples with nettle and chia seeds, the third one with pH 5.6–5.8 would be the largest one including samples with garlic, salt, thyme, caraway seed, almond lemon essences and beetroot additives. In last group with pH<5.6 would be samples with bell peppers and tomato additives. The average pH value of all samples was 5.77±0.01, but for control sample it was 6.35±0.01.

Table 3

<table>
<thead>
<tr>
<th>Added additive</th>
<th>Value</th>
<th>∆E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chia seeds</td>
<td>-4.3</td>
<td>4.6</td>
</tr>
<tr>
<td>Nettle</td>
<td>-19.7</td>
<td>19.7</td>
</tr>
<tr>
<td>Caraway-seeds</td>
<td>-7.5</td>
<td>7.7</td>
</tr>
<tr>
<td>Bell peppers</td>
<td>-11.1</td>
<td>14.7</td>
</tr>
<tr>
<td>Salt</td>
<td>-6.4</td>
<td>6.5</td>
</tr>
<tr>
<td>Beetroot</td>
<td>-27.0</td>
<td>35.8</td>
</tr>
<tr>
<td>Spinach</td>
<td>-13.2</td>
<td>13.8</td>
</tr>
<tr>
<td>Almond essence</td>
<td>-5.1</td>
<td>5.3</td>
</tr>
<tr>
<td>Lemon essence</td>
<td>-4.9</td>
<td>4.9</td>
</tr>
<tr>
<td>Thyme</td>
<td>-8.2</td>
<td>8.2</td>
</tr>
<tr>
<td>Tomato</td>
<td>-8.2</td>
<td>10.3</td>
</tr>
<tr>
<td>Garlic</td>
<td>-6.9</td>
<td>6.9</td>
</tr>
</tbody>
</table>
SHREDDED CARROTS QUALITY PROVIDING BY TREATMENT WITH HYDROGEN PEROXIDE

Ingrida Augspole1, Tatjana Rakcejeva2, Zanda Kruma2, Fredijs Dimins1

1Department of Chemistry, Faculty of Food Technology, Latvia University of Agriculture, Liela Street 2, Jelgava, Latvia
2Department of Food Technology, Faculty of Food Technology, Latvia University of Agriculture, Liela Street 2, Jelgava, Latvia

e-mail: ingrida.augspole@inbox.lv

Abstract

Carrots are a vegetable crop providing a source of important nutritional compounds, but their shelf-life, especially for shredded carrots, is not so long. Minimally processed carrots are very common in developed countries and are gaining popularity due to their convenience and freshness. Hydrogen peroxide (H2O2) is also a well-studied oxidant agent, directly toxic to pathogens. It has both bacteriostatic and bactericidal activity.

The purpose of the present research was to investigate H2O2 influence on shredded carrots chemical composition. Shredded carrots were treated with 0.5, 1.0 and 1.5% H2O2 water solution for 30±1 s, 60±1 s and 90±1 s. The major sugars (fructose, glucose and sucrose) were determined by applying the method of high performance liquid chromatography (HPLC). The total phenol content was determined spectrophotometrically according to the Folin-Ciocalteu method and antioxidant activity was determined using the DPPH2 (2,2-diphenyl-1-1-picrylhydrazyl radical) assay.

Significant influence of H2O2 water solution concentration and treatment time on analyzed shredded carrots quality parameters was found. It was detected that antioxidant activity decrease by approximately 28% in average in shredded carrots after treatment with disinfectant, total phenolic content by approximately 43% in average and total sugars by approximately 30% in average. For the maximal quality parameters preservation recommended H2O2 water solution concentration is 1.5% and treatment time 30±1 s. As a result the content of total phenolic decreases by 35%, antioxidant activity by 20%, total sugars by 19%. As well, the content of fructose decreases by 8%, glucose by 18% and sucrose by 33%.

Keywords: total phenols, antioxidant activity, sugars, H2O2.

Introduction

Vegetables are an important part of our diet. They provide, not only the major dietary fiber component of our food, but also a range of micronutrients, including minerals, vitamins and antioxidant compounds, such as carotenoids and polyphenols. The nutritional value of fruit and vegetables is often associated with their antioxidant capacities (Singha et al., 2012).

Fresh-cut carrots (Daucus carota L.) could be finding on the market place as: whole peeled (baby), sticks, and sliced, shredded, grated and diced. Carrot is considered one of the vegetables whose consumption, both fresh and processed, has increased over the past years due not only to the nutritional and health benefits this vegetable provides, but also to the introduction of new carrot-derived products (Augspole, Rakcejeva, 2013).

One of the procedures involved in fresh-cut fruit and vegetable processing is cutting the product into smaller pieces. This cutting action essentially is causing the tissue to endure wounding stress. Wounding will cause some physiological effects to the tissue and it is understood that a more severe cutting process will elicit a greater wounding response decreasing the quality of the produce (Surjadinata, Cisneros-Zevallos, 2012).

Along with carotenoids, phenolic compounds also reveal antioxidative properties in vegetables. Phenolic compounds, especially flavonoids, show various types of biological activity, but the most important is the antioxidative activity. Still, it should be emphasized that phenolic compounds, beside coumarin compounds and lignins, are also synthesized in external carrot tissue as a result of mechanical injuries during harvest. This causes negative changes in the quality of storage roots, such as enzymatic browning or deterioration of taste and aroma as a result of enzymatic oxidation of pigments and unsaturated fatty acids (Smoleń, Sady; 2009). Phenolic compounds can act as antioxidants by interfering with oxidation processes through chain-breaking reaction activities (primary oxidation) or through scavenging of free radicals (secondary oxidation). The antioxidant properties of phenolic compounds stimulate the need to design strategies to enhance its content in plant tissues (Heredia, Cisneros-Zevallos, 2009).

The content of total phenolics in carrots ranges from 539.76 to 395.35 mg 100 g−1 (GAE) gallic acid equivalents of dry weight (Augspole et al., 2012). Phenolic acids and isocoumarins, predominant phenolics in carrots, contribute to plant tissue’s defense mechanism against infections or injuries. Phenolic acid content in carrot peels may increase up to seven-fold when carrots are subjected to abiotic stress during postharvest handling and storage. Phenolic have also been associated with imparting bitter and sour tastes to stored and processed carrots and may result in color loss in processed strained carrots. The total content of phenolic acids in fresh carrots ranges from 77.2 mg kg−1 of fresh weight for yellow varieties to 74.6 mg kg−1 of fresh weight for purple varieties; in processed strained carrots the amount is between 24.9 and 156.2 mg kg−1 of dry weight (Socaciu, 2008).

Carrots, in particular, are noted for their rich antioxidants, especially β-carotene. In recent years, worldwide consumption of carrots has been steadily increasing because of their nutritional benefits. Carrots carry other potentially beneficial health effects, boasting anti-carcinogenic, anti-aging, antioxidant, and...
immune-boosting properties, as well as the pro-vitamin activity of some carotenoids, all of which add to their importance in the diet (Yena et al., 2008). In the specific case of carrots, the most important micronutrient is β-carotene, which is a lipid-soluble carotenoid. Its typical chemical structure, consisting of a polyene chain with 11 conjugated double bonds and a β-ring at each end of the chain, gives β-carotene some health related properties (Knockaert et al., 2013). Among the frequently used processes applied with the purpose of reducing/eliminating pathogens from food, heat-based treatments are the most efficient ones. Hydrogen peroxide (H₂O₂) is also a well-studied oxidant agent, directly toxic to pathogens. It has both bacteriostatic and bactericidal activity, also due to its capacity to generate other cytotoxic oxidizing species, such as hydroxyl radicals. At a concentration between 1 and 5%, hydrogen peroxide is generally used as sanitizer of some food contact surfaces and packaging materials in aseptic filling operations. The antimicrobial efficiency of hydrogen peroxide at higher concentrations (4–5%) is comparable to 100–200 ppm of chlorine treatment (Alexandre et al., 2012). Fresh cut fruits and vegetables have been very popular for the bioavailability of numerous vitamins, minerals and other phytochemicals. However, they may naturally contain a wide variety of bacteria, fungi and yeast species. Commercial or homemade fresh cut fruits and vegetables are prepared by some simple treatments such as washing, cutting, grating, shredding and packaging. Among these steps, washing may be considered as the most critical step since it removes the soil particles and reduces the microbial load from the surface. Antimicrobial effects of chlorine based sanitizers on fresh cut produce have been previously reported, because they have been widely used as washing solution for fresh cut products to eliminate the microorganisms (Tornuka et al., 2012).

After summarizing data form scientific literature it was concluded, that hydrogen peroxide has mainly microbiological effect on treated food products. But not complete data was found about hydrogen peroxide influence on carrots chemical composition especially on antioxidant activity, phenols and sugars content. Therefore future experiments are needed. The purpose of the present research was as follow – to investigate hydrogen peroxide influence on shredded carrots chemical composition.

Materials and Methods

Materials

The research was accomplished on serotinous ‘Nante’ carrot (Daucus carota L.) hybrids ‘Nante/Forto’ harvested in Zemga region (Latvia) in the first part of October 2012 and was immediately used for experiments. Carrots were rinsed under the tap water, than peeled and shredded using Philips Comfort HR 7605 device. Shredded carrots were treated with 0.5, 1.0 and 1.5% H₂O₂ water solution for 30±1 s, 60±1 s and 90±1 s. As a control sample non-treated carrots was analysed for comparison.

H₂O₂ water solution preparation

To prevent degrading of the hydrogen peroxide solutions were prepared by mixing food grade concentrated hydrogen peroxide 30 g 100 g⁻¹ (Peróxidos do Brasil Ltda, Curitiba, Brazil) with sterile deionized water; solution was prepared one minute before the treatment process (Delgado et al., 2012; Watson et al., 2007).

Total phenolic content

The total phenolic content of carrots was determined by using Folin-Ciocalteu assay. An aliquot (1 mL) of extracts or standard solution of gallic acid 20, 40, 60, 80 and 100 mg L⁻¹ was added to 25 mL volumetric flask, containing 9 ml of distilled deionized water. Reagent blank using distilled deionized water was prepared. One milliliter of Folin-Ciocalteu’s phenol reagent was added to the mixture and shaken. After 5 min, 10 mL of 7% Na₂CO₃ solution was added to the mixture. The solution was diluted to volume 25 mL with distilled deionized water and mixed. After incubation for 90 min at room temperature, the absorbance against prepared reagent blank was determined at 750 nm with spectrophotometer JENWAY 6300 (Baroworld Scientific Ltd., UK). Total phenolic content of carrots was expressed as mg gallic acid equivalents (GAE) 100 g⁻¹ in fresh weight (Baydar et al., 2006; Marinova et al., 2005). Then for result veracity a phenolic content of carrots was expressed as mg gallic acid equivalents (GAE) 100 g⁻¹ in dry matter.

Antioxidant activity

The antioxidant activity was measured by the DPPH radical method according to A.L.K. Faller and E. Fialho, 2010 (Faller, Fialho: 2010). The antioxidant reaction was initiated by transferring 0.5 mL of carrot extract into a sample cavity containing 3.5 mL of freshly prepared DPPH methanol solution (0.004 g DPPH to 100 mL methanol). After 30 min of incubation in the dark at room temperature, the absorbance was measured at 517 nm with spectrophotometer JENWAY 6300 (Baroworld Scientific Ltd., UK). Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. Each determination was performed in triplicate, and the results were expressed as mean ± SD. Inhibition of DPPH in percentage (I%) of each carrot sample was calculated from the decrease of the absorbance according to the relationship:

\[ I \% = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100 \]  

where \( A_{\text{blank}} \) is the absorbance of the control reaction (methanol–water with DPPH), and \( A_{\text{sample}} \) is the absorbance of a carrot sample (Faller, Fialho, 2010).

Sugars

The content of glucose, fructose and sucrose of carrots grown in Latvia is determined by applying the method of high performance liquid chromatography (HPLC).
The method is based on the fact that the chromatographic separation of glucose, fructose and sucrose is based on their delayed time differences. To 5±0.01 g of sample 20 mL of water was added into a 50 mL volumetric flask, heated for 20 min at 60 °C in a water bath and cooled to ambient temperature (20±2 °C). Then, 1 mL of Carrez I and 1 mL of Carrez II solutions were added and shaken. A volumetric flask was filled up with water till the mark and shaken well. First, extract was filtered through a membrane filter with pore size of 0.2 μm. Second, extract was placed in a vial and tested by HPLC Prominence (Shimadzu, Japan) equipped with Sypelcosil™ LC-NH2 column (250×4.6 mm, particle size – 5 μm) and autosampler SIL-20A. Sugars were detected with a refractive index detector RID-10A (Shimadzu); acquired data were processed using Shimadzu LabSolutions software (LCSolution Version 1.21 SP1). Acetonitrile: water (80:20 v/v) was used as eluent while column temperature was held at 30 °C. The flow rate was 1.0 mL min⁻¹. Injection volume of samples was 10 μL. Calibration curve was acquired after two repeated HPLC runs of seven standard solutions of reference compounds.

The chromatography data processing system fixes the composition of glucose, fructose, and sucrose in carrots by comparing the carrot chromatography with the chromatography of sugar standard-solution.

**Mathematical data processing**

Data are expressed as mean ± standard deviation; for the mathematical data processing p-value at 0.05 (Two Way analysis of variance, ANOVA), was used to determine the significant differences. In case of establishing statistically significant differences, homogeneous groups were determined by Tukey’s multiple comparison test the level of confidence α=0.05. The statistical analyses were performed using Microsoft Excel 2007.

**Results and Discussion**

**Total phenolic content**

The presence of phenolic compounds in carrots contributes to their sensory qualities, like colour, bitterness, or aroma. Therefore, the response of phenolic compounds could be used as a good indicator to evaluate the vegetables quality during processing and storage. Major phenols in carrots include chlorogenic, caffeic, and p-hydroxybenzoic acids along with numerous cinnamic acid derivatives. The different carrot tissues have similar composition, but the individual phenolic content differs and it decreases from the exterior (peel) to the interior (xylem). Moreover, the reported data may vary with the extraction method, the way to express the results, and other factors such as cultivars, post-harvest and processing conditions (Gonçalves et al., 2010).

In the present research significant influence (p<0.05) of H₂O₂ water solution concentration and treatment time on total phenolic content amount was found. The content of total phenolic content decreases equally by 43%, comparing with initial content of total phenolic, amount in non-treated shredded carrots (Fig. 1).

**Figure 1. Total phenolic content in carrots**

It was proved, that comparing the influence of different H₂O₂ concentrations on the content of total phenolic there are not significant differences (p=0.962). The similar tendency was observed analyzing the influence of treated time 30–90 s (p=0.793). Acquired results mainly could be explained with possible intensive oxidation processes occurring in carrots. Very similar data was detected of shredded carrots samples treated with 1.5% H₂O₂ water solutions for 30 and 60 s (Fig. 1), however, the content of total phenolic was higher as 292.85±0.02 and 288.54±0.08 (GAE) mg 100 g⁻¹ in dry matter respectively.

**Antioxidant activity**

Assessments of antioxidant properties of natural compounds are very important because of their uses in medicine, food and cosmetics (Mishra et al., 2012). During the last few years, the study of antioxidant capacity has received much attention, mainly due to the growing interest in the efficiency and function of natural antioxidants in food and biological systems. The antioxidative reactions of free radicals, which are molecules with unpaired electrons, are thought to contribute too many health problems, including cancer, cardiovascular diseases, and inflammatory problems and aging. Antioxidants are agents that, in one way or another, restrict the deleterious effects of these oxidant reactions, either scavenging free radical (eliminating them without generating more radical-induced damage) or other effects (i.e. preventing radical formation) (Fernandez-Orozco et al., 2011).

In the present experiments significant influence (p<0.05) of hydrogen peroxide concentration and shredded carrots processing time on antioxidant activity with disinfectant was established. It is indicate, that antioxidant activity decrease by 28% in average in shredded carrots after treatment with H₂O₂ disinfectant (Fig. 2).

After mathematical data processing was proved, that comparing the influence of different H₂O₂ concentrations and treated time 30–90 s on the antioxidant activity there are not significant differences significant difference (p=0.617) was not established in
antioxidant activity of with hydrogen peroxide treated shredded carrots. Obtained results are very similar with results on total phenolic contents (Fig 1).

![Antioxidant activity graph](image)

**Figure 2. Carrots antioxidant activity**

Maximally total antioxidant activity of shredded carrots is possible to maintain by processing with 1.5% hydrogen peroxide water solution for 30s, as a result antioxidant activity value was 14.92±0.03%, while, the total antioxidant activity of non-processed shredded carrots was 18.70±0.04%. Therefore, close interconnection (r=0.953) between total phenolic compounds and total antioxidant activity was detected in the present research.

**Sugars**

One of the most important qualities of vegetables is their sweetness, closely related to the soluble sugar content (Ozaki et al., 2009). Carrot is mainly constituted by water (approximately 90% of fresh weight) and carbohydrates, which account for 5% of carrot edible portion. In addition to terpenoids, carbohydrates have been reported to be one of the most important sensory indicators for consumer appreciation of this vegetable. As it is known, fructose, glucose and sucrose are the major sugars in carrot and extensive research has been published on their content in carrots of different variety and / or submitted to different processing and storage conditions (Soria et al., 2009).

In the present research negative influence of hydrogen peroxide on total sugar content and fructose, glucose and sucrose content in shredded carrots was detected (Table 1).

In the research obtained results demonstrate, there are not found significant differences (p>0.05) in total sugar content if shredded carrots was treated by 0.5% and 1.0% H₂O₂ water solution for 30–90 s. As a result the content of total sugars decreased by 33% in average compared to the control sample.

Significant differences (p<0.05) in sugars content was found between shredded carrots samples treated with 1.5% H₂O₂ water solution for 30 and 90 s and for 60 and 90 s (Table 1). The content of total sugars decreased by 30% in average, if carrots were treated with different H₂O₂ concentrations for treated time 30–90 s compared to the non-processed shredded carrots. Mainly acquired results could be explained with possible oxidation processes influenced by hydrogen peroxide.

Table 1

<table>
<thead>
<tr>
<th>H₂O₂ water solution, *%</th>
<th>Time, s**</th>
<th>Fructose, g 100 g⁻¹</th>
<th>Glucose, g 100 g⁻¹</th>
<th>Sucrose, g 100 g⁻¹</th>
<th>Total sugars, g 100 g⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0.72±0.04</td>
<td>0.55±0.02</td>
<td>4.51±0.01</td>
<td>5.06±0.02</td>
</tr>
<tr>
<td>0.5</td>
<td>30±1</td>
<td>0.46±0.01</td>
<td>0.47±0.01</td>
<td>2.75±0.02</td>
<td>3.68±0.01</td>
</tr>
<tr>
<td></td>
<td>60±1</td>
<td>0.26±0.01</td>
<td>0.45±0.02</td>
<td>2.69±0.01</td>
<td>3.40±0.02</td>
</tr>
<tr>
<td></td>
<td>90±1</td>
<td>0.41±0.02</td>
<td>0.31±0.02</td>
<td>2.27±0.01</td>
<td>2.99±0.02</td>
</tr>
<tr>
<td>1.0</td>
<td>30±1</td>
<td>0.50±0.02</td>
<td>0.66±0.01</td>
<td>2.54±0.02</td>
<td>3.70±0.02</td>
</tr>
<tr>
<td></td>
<td>60±1</td>
<td>0.44±0.04</td>
<td>0.13±0.01</td>
<td>2.45±0.01</td>
<td>3.02±0.01</td>
</tr>
<tr>
<td></td>
<td>90±1</td>
<td>0.65±0.01</td>
<td>0.26±0.01</td>
<td>2.48±0.04</td>
<td>3.39±0.01</td>
</tr>
<tr>
<td>1.5</td>
<td>30±1</td>
<td>0.66±0.01</td>
<td>0.45±0.01</td>
<td>3.01±0.02</td>
<td>4.12±0.01</td>
</tr>
<tr>
<td></td>
<td>60±1</td>
<td>0.26±0.01</td>
<td>0.19±0.03</td>
<td>2.76±0.06</td>
<td>3.21±0.01</td>
</tr>
<tr>
<td></td>
<td>90±1</td>
<td>0.37±0.01</td>
<td>0.51±0.01</td>
<td>2.09±0.01</td>
<td>2.97±0.01</td>
</tr>
</tbody>
</table>

*concentration  
**treatment time

**Conclusions**

In the present research significant influence (p<0.05) of H₂O₂ water solution concentration and treatment time on analyzed shredded carrots quality parameters was found. It was detected that antioxidant activity decrease by approximately 28% in average in shredded carrots after treatment with disinfectant, total phenolic content by approximately 43% in average and sugars by approximately 30% in average compared to the non-processed shredded carrots. For the maximal quality parameters preservation recommendable H₂O₂ water solution concentration is 1.5% and treatment time 30±1 s. In this treatment the
content of total phenolic compounds decrease by 35%, antioxidant activity by 20%, total sugars by 19%. As well, the content of fructose decreases by 8%, of glucose by 18% and of sucrose by 33%.

Acknowledgment
The research partly has been prepared within the framework of the ESF Project “Formation of the Research Group in Food Science”, Contract No. 2009/0232/IDP/ 1.1.1.2.0/09/APIA/VIAA/122.

References
EFFICIENCY OF THE ADDITIONAL FERTILIZATION WITH NITROGEN FERTILIZERS GROWING CARROT OF EXCEPTIONAL QUALITY AND ITS INFLUENCE ON PRODUCTION STORAGE

Ona Bundinienė, Vytautas Zalatorius, Danguolė Kavaliauskaitė, Roma Starkutė
Institute of Horticulture Lithuanian Research Centre for Agriculture and Forestry, LT-54333, Babtai, Kaunas distr.,
e-mail: o.bundiniene@lsd.lt

Abstract
Plant nutrition, among other agro-technical measures most influences yield, quality and production storability. The aim of investigations – instilling new technological elements of the growing of root-crop vegetables of exceptional quality to choose for the additional fertilization the most suitable nitrogen fertilizer and its rate in order the soil, environment was polluted as little as possible and the qualitative production was obtained. There were grown carrot ‘Nerac’ F₁ and ‘Tito’. There was investigated the influence of nitrogen fertilizer used for additional fertilization (ammonium nitrate and calcium nitrate) and their rates (N₀ and N₁₅) on carrot yield, and its impact on production storage. Additional fertilizations were carried out at 2–4 carrot leaves stage. Carrot were grown on profiled surface, sowing scheme 62+8, there were sown 1 ml. until. ha⁻¹ germinable seeds. Before sowing the field was fertilized with complex fertilizers. Carrots during vegetation period were additionally fertilized through leaves for three times applying soluble complex fertilizers. Experiments were carried out on sandy loam on light loam Calci(car)Ephipogleic Luvisol (LVg-p-w-cc). Data of investigations showed that carrot hybrid was more productive than cultivar. Calcium nitre was more effective both to hybrid and cultivar. Vegetables fertilized with it was preserved better than these fertilized with ammonium nitrate.

Keywords: nitrogen fertilizer, exceptional quality.

Introduction
The intensity of agricultural activities determines the level of maintenance of cultural landscapes, biodiversity, and the cultural identity of an area (Li et al., 2013). Success in vegetable culture in a temperate climate has been due to proper mineral nutrition of vegetables, because plant nutrition, among other agro-technical measures most influences yield, quality and production storability (Biesiada et al., 2011; Kant et al., 2011). However, the surplus of supply nutrients, especially nitrogen, may have an adverse effect on the environment as well as quality of the crops. More than 50% of N applied is effectively used by plants, but a large part is lost by leaching and it can lead the contamination of ground and surface water (Fageria et al. 2008; Wang et al., 2008). Optimization of technologies for growing plants is a method to increase plant productivity and to obtain quality yields (Cai, Ge, 2004; McAllister et al., 2012). The data of Cameron (2013) has shown that careful management of temperate systems using best management practices and newly developed technologies can increase the sustainability of agriculture and reduce its impact on the environment. In order to avoid negative consequences on the environment and to grow the production of exceptional quality, the amount of nitrogen scattered during vegetation was limited.

Carrot (Daucus carota L.) is the important vegetable crop, grown in temperate climate zone, which includes Lithuania. The objective of the study – instilling new technological elements of the growing of root-crop vegetables of exceptional quality to choose for the additional fertilization the most suitable nitrogen fertilizer and its rate in order the soil, environment was polluted as little as possible and the qualitative production was obtained.

Materials and methods
Experiments were carried out in the Institute of Horticulture in 2010 – 2012, on sandy loam on light loam Calci(car)Ephipogleic Luvisols (LVg-p-w-cc). Soil analysis in spring, before N fertilization, was carried out. The soil is characterized by the average content of humus (2.38–2.67), pH neutral to slightly alkaline (7.1–7.7) and by the presence of large amounts of Ca and Mg, which gives more favourable physical-chemical water and soil properties. Mineral nitrogen content in the layer 0–60 cm was low to average (44.2–87.7 mg kg⁻¹), while there was a good stock of soluble phosphorus (P₂O₅ = 166–262 mg kg⁻¹) and potassium (K₂O = 141–184 mg kg⁻¹).

The carrot ‘Nerac’ F₁ and ‘Tito’ (factor B) were grown on the profiled surface, 1.0 ml. ha⁻¹ viable seed. Both the other cases the sowing scheme were 62+8 cm. Scheme of the fertilization (factor A): 1. Basic fertilization (handed before presowing tillage with additionally foliar application (Basic – B); 2. B+calcium nitrate N₁₅(B+CN N₁₅); 3. B+calcium nitrate N₁₅(B+CN N₁₅); 4. B+ammonium nitrate N₀(B+AN N₀); 5. B+ammonium nitrate N₁₅(B+AN N₁₅). Soil fertilization was carried out with complex mineral fertilizers using a ratio N₈₄P₈₀K₁₀₀:₄. Fertilizers were applied before sowing of carrot. Nitrogen fertilizer for additional fertilization, introduced as ammonium nitrate (34% N, NO₃–N 17%, N-NH₄ 17%). and calcium nitrate (15.5% N, NO₃–N 14.4%, N-NH₄ 1.1 % and 26.4 % CaO), was applied once during the growing season when carrot was at 2–4 leaf stage. Carrot during vegetation period were additionally fertilized through leaves for three times applying soluble complex fertilizers: twice with Ferticare 14 11 25 with microelements, 5 kg ha⁻¹ by adding Delfan, 2 L ha⁻¹, once Ferticare 6 14 30 with microelements, kg ha⁻¹.
adding Final K, 2 L ha⁻¹. Experiments were conducted every year in 4 replications in systematic order. Area of record plot = 6.2 m².

Work of plant supervision was carried out according to vegetable growing technologies accepted in LIH, observing requirements of growing of exceptional quality production, where the use of nitrogen and pesticides is limited. Carrot yield was gathered, when vegetables reached technical maturity, classifying into marketable and nonmarketable. When harvesting according to the variants, with three replications, there were taken samples for biochemical investigations and 12–15 kg samples for root-crop storability investigations. Root-crops were stored in freezer, under stable temperature (-1--2°C) and relative humidity (85--90%), in the storage houses of Institute of Horticulture, LRCAF. Root-crop storability was inspected after 3 and 7 months, classifying well-preserved and diseased (rotten, partly rotten and wilted, i.e. not suitable for usage) red beet root-crops and establishing the natural loss, i.e., drying.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Average monthly air temperature at the experiment site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Month</td>
<td>Air temperature °C</td>
</tr>
<tr>
<td></td>
<td>2010</td>
</tr>
<tr>
<td>May</td>
<td>13.5</td>
</tr>
<tr>
<td>June</td>
<td>14.6</td>
</tr>
<tr>
<td>August</td>
<td>19.9</td>
</tr>
<tr>
<td>September</td>
<td>17.8</td>
</tr>
<tr>
<td>October</td>
<td>9.9</td>
</tr>
<tr>
<td>Average</td>
<td>13.2</td>
</tr>
</tbody>
</table>

Investigations of carrot biochemical composition were carried out at the Laboratory of Biochemistry and Technology, Institute of Horticulture, LRCAF. There was established: dry matter (DM) – gravimetrically, after drying out at the temperature of 105±2 °C up to the unchangeable mass (Food analysis, 1986), dry soluble solids (DSS) – with refractometer (digital refractometer ATAGO) (AOAC, Official..., 1990b), sugars – by AOAC method (AOAC, Official..., 1990a), nitrates – by potentiometrical method with ion selective electrode (Metod. nurod., 1990), carotenes – spectrophotometrically (Davies, 1976).

Analysis of variance was performed using the ANOVA statistical package with two-way factors. Treatment means were compared using Fisher’s protected least significant difference (LSD) test at p≤0.05. Contrasts were used to determine the effect of fertilization rates on yield, yield biochemical composition and quality of storage.

Meteorological conditions during the years of the experiment varied significantly (Tables 1, 2). The year 2010 was the colder, but with the higher temperature and higher amount of precipitation in July and August, as compared to multiannual.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Average monthly precipitation at the experiment site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Month</td>
<td>Precipitation, mm</td>
</tr>
<tr>
<td></td>
<td>2010</td>
</tr>
<tr>
<td>May</td>
<td>71.4</td>
</tr>
<tr>
<td>June</td>
<td>72.6</td>
</tr>
<tr>
<td>August</td>
<td>119.2</td>
</tr>
<tr>
<td>September</td>
<td>105.4</td>
</tr>
<tr>
<td>October</td>
<td>51.0</td>
</tr>
<tr>
<td>Average</td>
<td>70.1</td>
</tr>
</tbody>
</table>

The highest and relatively uniform mean temperature during the whole season was in 2011. The year 2011 had the highest total precipitation, with especially humid July and August, but dry June. In 2012 air temperature was slightly higher than the multiannual average, but June and August were cool and June and August were wet, while July was hot and wet.

Results and Discussion

Using nitrogen in both variants, irrespective cultivars, forms and rates, carrot marketable yield increased averagely by 6.3 t ha⁻¹ or 13.9%. The output of marketable yield increased correspondingly 9.2% (Fig. 1). Data of investigations carried out in various countries with various plants (Chohura, Kolota, 2009; Biesiada et al., 2011; Toivonen, Hodges, 2011) show that nitrogen determines the growth and productivity of plant most of all. According to the data of Zdravkovic et al. (1997), carrot fertilized with manure produced 48.4 t ha⁻¹, with calcium ammonium nitrate – 41.5 t ha⁻¹ and their mixture – 41.5 t ha⁻¹ of yield. The output of marketable yield is important parameter, which determines fertilizer suitability and corresponds to one of the main requirements of the optimal yield (Suojala, 2000; Zalatorius, Viškelis, 2005). Additionally fertilizing carrots with nitrogen at 2–4 leaf stage, marketable yield of cultivar ‘Nerac’ F₁ averagely increased 11.7%, the output of marketable yield – 3.4%. At the same time the yield of carrot cultivar ‘Tito’ increased 17.4%, output – 9.4%. The vegetable yields were not increased continuously with N rate increase, and oversupply of N reduced the plant growth, leading to a yield decline (Wang, Li, 2003). The data of our investigations showed that applying bigger nitrogen rate for additional fertilization the significant additional yield wasn’t obtained – comparing with the case when smaller nitrogen rate for carrot fertilization was used. Both calcium nitrate and ammonium nitrate applied for additional fertilization, irrespective of the rates of nitrogen and cultivars, were equally effective for carrots. The best yields were obtained additionally using 30 kg ha⁻¹ of nitrogen.
When the rate of nitrogen was decreased, marketable yield ‘Nerac’ F₁ decreased by 6.0 t ha⁻¹ or 11.7%, but marketable yield of cultivar ‘Tito’ slightly increased. The output of marketable yield was slightly smaller fertilizing with bigger nitrogen rate, but significant differences between the variants weren’t noted. Data of study Kolota et al. (2007) showed that application of ammonium nitrate, calcium nitrate and Entec 26 gave similar to each other and considerable better results than ammonium sulphate.

Dry matter and dry soluble solids, carotenes, nitrates and phenols in carrot root-crops. Paoletti et al. (2012) also indicate that production systems do not affect the nutritional quality of carrot. The amounts of dry soluble so lids, as noted Suojala (2000), can increase when yield gathering is delayed. Slightly bigger amount of dry matter and dry soluble solids as shown in our studies was found in root-crops of cultivar ‘Tito’. Big amount of sugars and carotenes, especially β-carotene, and small amount of nitrates are important carrot quality indices (Gajewski et al., 2009, 2010). Arscott and Tanumihardjo (2010) observed that their amounts depend on growth conditions, genotype and fertilization.

**Table 3**

**Influence of different nitrogen fertilizers and rates on biochemical composition of exceptional quality carrot**

<table>
<thead>
<tr>
<th>Fertilization (fact A)</th>
<th>Biochemical composition (fact B)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Hybrid ‘Nerac’ (factor B)</td>
<td>11.5</td>
</tr>
<tr>
<td>B+CN N₃₀</td>
<td>11.1</td>
</tr>
<tr>
<td>B+CN N₁₅</td>
<td>11.2</td>
</tr>
<tr>
<td>B+AN N₃₀</td>
<td>11.3</td>
</tr>
<tr>
<td>B+AN N₁₅</td>
<td>11.3</td>
</tr>
<tr>
<td>Cultivar ‘Tito’ (factor B)</td>
<td>11.8</td>
</tr>
<tr>
<td>B+CN N₃₀</td>
<td>11.5</td>
</tr>
<tr>
<td>B+CN N₁₅</td>
<td>11.7</td>
</tr>
<tr>
<td>B+AN N₃₀</td>
<td>11.9</td>
</tr>
<tr>
<td>B+AN N₁₅</td>
<td>11.7</td>
</tr>
<tr>
<td>Average fertilization (Factor A)</td>
<td>11.6</td>
</tr>
<tr>
<td>B+CN N₃₀</td>
<td>11.3</td>
</tr>
<tr>
<td>B+CN N₁₅</td>
<td>11.4</td>
</tr>
<tr>
<td>B+AN N₃₀</td>
<td>11.6</td>
</tr>
<tr>
<td>B+AN N₁₅</td>
<td>11.5</td>
</tr>
<tr>
<td>Average cultivars (factor B)</td>
<td>11.3</td>
</tr>
<tr>
<td>Cultivar</td>
<td>11.7</td>
</tr>
<tr>
<td>LSDᵦᵦᵦ(A)</td>
<td>0.3</td>
</tr>
<tr>
<td>LSDᵦᵦᵦ(B)</td>
<td>0.2</td>
</tr>
<tr>
<td>LSDᵦᵦᵦ(AxB)</td>
<td>0.42</td>
</tr>
</tbody>
</table>

1 – dry matter, %, 2 – dry soluble solids, %, 3 - sugars, %, 4 – nitrates, mg kg⁻¹, 5 – carotenes, mg 100 g⁻¹

Our studies have shown that the cultivar did not affect the sugar and carotene content in the roots, and additional nitrogen fertilization slightly increased the sugar content. Carotenes from additional nitrogen fertilizer use declined. Nitrogen fertilizer forms and rates had no significant effect on sugar and carotene

**Figure 1.** Influence of different nitrogen fertilizers and rates on marketable yield and output of marketable yield of exceptional quality carrot

Fertilization: 1 – Basic fertilization (B), 2 – B + CN N₃₀, 3 – B + CN N₁₅, 4 – B + AN N₃₀, 5 – B + AN N₁₅

Aytoko (2004) indicate that in carrot there can be 8–12% of dry matter, 6–8% of sugars, 9–12 mg% of carotene, also potassium and microelements – boron and iodine. Ayzay et al. (2007), affirm that amounts of dry matter fluctuated in wide limits – from 6.40% to 11.43%; nitrates – from 8.1 mg kg⁻¹ to 509 mg kg⁻¹. Their concentration in vegetables changes dependently on many factors: vegetable type and cultivar (Seljåsen et al., 2013, Chohura, Kolota, 2009; Roźek et al., 2000), soil, meteorological conditions (Rubatzky et al. 1999). Data of our investigations show that the amounts of dry matter and dry soluble solids fluctuated in very narrow limits and fertilization with nitrogen little influenced their changes (Table 3). Our present studies confirmed Polish (Pokluda, 2006) and Lithuanian (Pekarskas, Bartashevičienė, 2009; Karklelienė et al. 2007) investigators obtain data. They point out that differentiated soil fertilization with nitrogen doesn’t significantly influence the amount of nitrogen.
content. Too intensive fertilization, especially with nitrogen, according to many researchers (Ayaz et al., 2007; Kona 2006; Santamaria, 2005; Smoleń et al., 2011; Wang et al., 2008; Zeka et al., 2014) can cause unsuitable increases in some plants of nitrates, sugars and decreases of dry soluble solids, ascorbic acid (vitamin C), calcium and magnesium. There were found big differences between nitrates accumulation in different vegetable species (Gajewski et al., 2007). Our research has shown that tested cultivars did not affect the amount of nitrates in the roots. In the grown experiments in carrot root-crops irrespective cultivars there were 365.1 mg kg⁻¹ (Table 3). Fertilizing with nitrogen the amount of nitrates increased 13.7 mg kg⁻¹. The least amount of nitrates in root-crops accumulated in carrot fertilized with calcium nitrate.

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Influence of different nitrogen fertilizers and rates on storability of exceptional quality carrot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fertilization (factor A)</td>
<td>A</td>
</tr>
<tr>
<td>Basic (B)</td>
<td>43.5</td>
</tr>
<tr>
<td>B + CN N₃₀</td>
<td>53.4</td>
</tr>
<tr>
<td>B + CN N₁₅</td>
<td>48.4</td>
</tr>
<tr>
<td>B + AN N₃₀</td>
<td>51.2</td>
</tr>
<tr>
<td>B + AN N₁₅</td>
<td>48.6</td>
</tr>
</tbody>
</table>

| Cultivar 'Tito' (factor B) | | |
| Basic (B) | 28.9 | 5.8 | 7.4 |
| B + CN N₃₀ | 34.3 | 32.0 | 8.4 |
| B + CN N₁₅ | 35.1 | 31.4 | 5.9 |
| B + AN N₃₀ | 34.4 | 31.7 | 6.1 |
| B + AN N₁₅ | 32.5 | 30.8 | 5.9 |

| Average fertilization (factor A) | | |
| Basic (B) | 36.2 | 32.5 | 7.2 |
| B + CN N₃₀ | 43.8 | 41.9 | 6.9 |
| B + CN N₁₅ | 41.7 | 38.2 | 6.0 |
| B + AN N₃₀ | 42.8 | 40.0 | 5.8 |
| B + AN N₁₅ | 40.6 | 38.3 | 5.5 |

| Cultivars (factor B) | | |
| Hybrid | 49.0 | 49.9 | 5.8 |
| Cultivar | 33.0 | 30.4 | 6.8 |
| LSD₀.₀5(A) | 4.7 | 4.7 | 1.2 |
| LSD₀.₀5(B) | 2.3 | 2.3 | 0.8 |
| LSD₀.₀5(AxB) | 7.0 | 7.0 | 2.4 |

A – after short-time storage; B – after long-term storage; A₁, B₁ – marketable production, t ha⁻¹; A₂, B₂ – sick, %; A₃, B₃ – natural losses

Vegetable storability is influenced by climatic conditions, soil properties, cultivars, fertilization and forms of fertilizers, as well as the time of harvesting (Rożek et al., 2000; Fikseliová et al., 2010; Wrzodak et al., 2012). To store carrot is more difficult than other root-crop vegetables, because their root-crop, as observed Autko (2004) have a thin epithelium tissue (4–8 layers of periderma, while potato – 9-11 layers), which during yield gathering with mechanical means very often is injured. Therefore water is evaporated more intensively and root-crops of carrot quickly wilt, and wilted are less resistant to diseases, but small mechanical injures root-crop is able to “heal up”. According to Suslov et al. (2009), storage of carrots for a pro-longed period of time at temperatures above 2 °C results in heavy sprouting of the tops and lateral roots. When carrot cultivar ‘Nerac’ F₁ was stored for short time (up till New Year) marketable production comprised averagely 49.0 t ha⁻¹, and after long-time storage (up till May) – 45.9 t ha⁻¹, and cultivar ‘Tito’ correspondingly 33.0 and 30.4 t ha⁻¹ (Table 4). Storage losses correspondingly were 4.8% and 5.9%, and after long-term storage – 9.2 and 12.3%. Losses of cultivar short-term and long-term storage were slightly bigger than these ones of hybrid. Marketable production of carrot fertilized with nitrogen fertilizers after short-time storage, irrespective of cultivars, increased on the average 6.0 t ha⁻¹ or 16.7%, after long-time storage – 7.1 t ha⁻¹ or 21.8%. This is due to the fact that nitrogen fertilizer increased yields. Nitrogen fertilizer forms and rates used for additional fertilization did not affect the storability quality of carrot. Wrzodak et al. (2012) observes that there were marked differences in storage life depending on the cultivar, cultivation method and storage period.

Conclusions

The best marketable yield was obtained additionally using 30 kg ha⁻¹ of nitrogen in form of calcium nitrate. The marketable yield of carrot ‘Nerac’ F₁ was 56.4 t ha⁻¹, cultivar ‘Tito’ – 36.3 t ha⁻¹. The amounts of dry matter and dry soluble solids fluctuated in very narrow limits and fertilization with nitrogen little influenced their changes. Slightly bigger amount of dry matter and dry soluble solids as shown in our studies was found in root-crops of cultivar ‘Tito’. Additional nitrogen fertilization slightly increased sugar content. Carotenes from additional nitrogen fertilizer use declined. Fertilizing with nitrogen the amount of nitrates increased 13.7 mg kg⁻¹. The least amount of nitrates in root-crops accumulated in carrot fertilized with calcium nitrate. Used for additional fertilization nitrogen fertilizer forms and rates did not affect the storability quality of carrot. Losses of cultivar short-term and long-term storage were slightly bigger than these ones of hybrid. The marketable production after short-time storage irrespective of cultivars increased on the average 6.0 t ha⁻¹ or 16.7%, after long-time storage – 7.1 t ha⁻¹ or 21.8%.
References


IMPACT OF THE DEGREE OF MATURITY ON APPLE QUALITY DURING THE SHELF LIFE

Karina Juhnevica-Radenkova¹,², Liga Skudra², Mara Skrivele¹, Vitalijs Radenkovs¹,², Dalija Seglina¹

¹ Latvia State Institute of Fruit-Growing, 1 Grauda street, Dobeles, Latvia, e-mail: vitalijs.rad@inbox.lv
² Department of Food Technology, Faculty of Food Technology, Latvia University of Agriculture, 2 Liela street, Jelgava, Latvia

Abstract

The main objective of this work was to determine optimum harvest date in apples, which were kept in long term as well as shelf-life storage (supermarket, T=18 °C). The effect of storage conditions and inhibitor of ethylene 1-methylcyclopropene (1-MCP) treatment on ethylene production and quality of apples stored in normal and controlled atmosphere conditions was evaluated during one successive season 2012/2013. Experiments were carried out at the Latvia State Institute of Fruit-Growing. Treated and untreated (control) apples were stored for six months in the cold storage rooms at 2±0.5 °C under normal atmosphere (NA) conditions or controlled atmosphere (CA) with 1.0% O₂, 2.0% CO₂, 97% N₂ and 1.5% O₂, 2.5% CO₂, 96% N₂. Directly after storage and after shelf-life (additionally 14 days at 18 °C) internal ethylene concentration was determined. For estimation of apple quality sensory evaluation and density were chosen. The obtained results give the possibility to conclude that almost all cultivars which are early harvested were suitable for storage in modified atmosphere. The apple storage in modified atmosphere gives the opportunity to extend the apple shelf-life period more than two weeks simultaneously without any qualitative changes.

Keywords: apples, quality, shelf-life, storage.

Introduction

Consumers mainly associate quality of apple with their firmness, juiciness, and sweetness. The softness of apples (mealoiness) characterise a fruit on with low quality and suggest that fruits were stored too long (Galmarini et al., 2013). The shelf-life period of apples is determined by number of indicators. It has been reported by Gregersons from University of Illinois USA that apple expiration date mainly depends from chemical composition, especially from content of water and sugar (Gregersons, 2009), another authors suggested that it’s depended from thickness of the wax layer and from the cultivars (Soliva-Fortuny et al., 2002). Winter cultivars which contain lower amount of water comparing with summer cultivars, always kept longer. There are a lot of factors which can affect the apples expiration date. Basically significant affect renders the ripeness of apples, for example ‘Granny Smith’ cultivar which was harvested slightly green and stored in controlled modified atmosphere for about a year, will be kept in shelf-life approximately 6–8 weeks, however the apple will not have a strong taste and aroma. According to Raffo and colleagues the apple storage in controlled modified atmosphere extend the expiration date up to 60 days, on the other hand it affect the sensory qualities of the products (Raffo et al., 2009). This type of storage not only delay the aging process, but also suppress the volatile organic compounds (esters, aldehydes, alcohols and terpenes), which is responsible for the fruit aroma development. Fruit quality on shelves is also dependent from the storage temperature and air relative humidity in the storage chamber. Traditionally the storage camera (refrigerator) can impair the texture of apples, as a result, during the expiration time these fruits can promptly become soft and mealy (Hui 2010). One of the most important indicators during the storage of apples is the placement in the shop. During the apple respiration processes the ethylene production increase, which accelerate the aging of the apple, and if they are stored near by the fruits which are also produce ethylene (pears, peaches, nectarines, plums, tomatoes, avocados, bananas, kiwi, mango and apricot), the ripening process become up to 10 times intensively, as a result maximal expiration date for this product will be 1–2 weeks. One of the possibility how can prolong the expiration date of fruit is 1-MCP treatment with the aim to delay the ethylene production. It gives the opportunity to extend the shelf-life period up to 4–6 weeks. However, there is still a risk that during the storage a receptor of ethylene will recovered and the cells will become susceptible to ethylene molecules (Abadi et al., 2009). Scientist from Spain Valera (2008) has determined the maximal shelf-life period for apples after storage in modified atmosphere. It has been reported that after 61 day storage of apples in a shop at 20 °C temperature, consumer evaluation showed that the quality did not changed significantly, while after 70 days of storage apples became softer. The aim of the work was determine optimal harvest date in apples, which are kept in long term as well as shelf-life storage (supermarket, T=18 °C).

Materials and Methods

The research has been conducted at the Latvia State Institute of Fruit-Growing (LSIFG) with the seven different commercial apple cultivars: ‘Auksis’, ‘Orlik’, ‘Gita’ (autumn), ‘Antej’, ‘Beloruskoje Malinovoje’, ‘Sinap Orlovskij’, ‘Zarja Alatau’ (winter). Apples were preserved in controlled modified atmosphere T=2±1 °C for six months, and two weeks stored in natural atmosphere (shelf-life) T=18±1 °C. The storage time was choosen accordingly with the report from Гудковский (2012). ULO1 chamber with gas content O₂=1.00%, CO₂=2.00%, and ULO2 chamber with gas content O₂=1.50%, CO₂=2.50% was used for preservation in controlled modified environment, and control samples...
were stored in cooling chamber at temperature 2±1 °C with relative humidity 85%. Treatment with 1-MCP was performed when fruit were ready for consumption. 1-MCP was obtained from Hangzhou Ruijiang Chemical Co., Ltd. 1-MCP was dissolved in warm water at ratio 1 : 30 in a sealed container. The container with 1-MCP was placed into a hermetically closed fruit processing cabinet; then the plug was opened and the treatment was performed for 24 h at 18±1 °C. The final concentration of 1-MCP in processing cabinet was 0.625 µL·L⁻¹.

For optimal long term storage the apples with following parameters were chosen (Table 1 and 2).

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Concentration of ethylene, µL h⁻¹ L⁻¹ kg⁻¹</th>
<th>Firmness, kg cm⁻²</th>
<th>The ration of soluble solids / titratable acidity, °Brix / %</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Auksis’</td>
<td>0.174</td>
<td>9.400</td>
<td>18.177</td>
</tr>
<tr>
<td>‘Orlik’</td>
<td>0.181</td>
<td>8.490</td>
<td>13.301</td>
</tr>
<tr>
<td>‘Gita’</td>
<td>0.170</td>
<td>6.070</td>
<td>10.989</td>
</tr>
<tr>
<td>‘Antej’</td>
<td>0.221–0.252</td>
<td>6.320–6.440</td>
<td>16.879–17.530</td>
</tr>
<tr>
<td>‘Beloruskoje Malinovoje’</td>
<td>0.187–0.204</td>
<td>8.640–6.770</td>
<td>11.432–13.573</td>
</tr>
<tr>
<td>‘Sinap Orlovskij’</td>
<td>0.177–0.188</td>
<td>8.640–8.810</td>
<td>13.561–15.377</td>
</tr>
<tr>
<td>‘Zarja Alatau’</td>
<td>0.187–0.215</td>
<td>6.050–6.920</td>
<td>17.765–19.077</td>
</tr>
</tbody>
</table>

Data were obtained in our previous investigations.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Iodine starch test (1–10)</th>
<th>Streif’s index</th>
<th>De Jager’s index (PFW-1)F * (11 – S) / R</th>
<th>FARS index</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Auksis’</td>
<td>5.0</td>
<td>0.167</td>
<td>5.004</td>
<td>0.103</td>
</tr>
<tr>
<td>‘Orlik’</td>
<td>4.0</td>
<td>0.192</td>
<td>5.383</td>
<td>0.160</td>
</tr>
<tr>
<td>‘Gita’</td>
<td>5.0</td>
<td>0.116</td>
<td>3.489</td>
<td>0.110</td>
</tr>
<tr>
<td>‘Antej’</td>
<td>4.5–5.0</td>
<td>0.109–0.128</td>
<td>3.27–3.758</td>
<td>0.072–0.085</td>
</tr>
<tr>
<td>‘Beloruskoje Malinovoje’</td>
<td>5.0</td>
<td>0.133–0.187</td>
<td>3.990–5.598</td>
<td>0.100–0.151</td>
</tr>
<tr>
<td>‘Sinap Orlovskij’</td>
<td>4.0–4.8</td>
<td>0.152–0.198</td>
<td>4.524–5.546</td>
<td>0.117–0.162</td>
</tr>
<tr>
<td>‘Zarja Alatau’</td>
<td>4.5–6.7</td>
<td>0.073–0.127</td>
<td>2.098–3.724</td>
<td>0.047–0.087</td>
</tr>
</tbody>
</table>

Data were obtained in our previous investigations.

Determination of physicochemical parameters

Soluble solids content
The soluble solids content was determined according to LVS EN 12143:2001 standard.

Titratable acidity
The changes in acidity characterize the degree of apples ripeness. Titratable acid content is determined according to AOAC 942.15 standard.

Apple density
The changes in the fruit pulp density describe the degree of ripeness of apple. The density was measured with a digital penetrometer. Procedure and data collection was done according to LVS EN 1131:2001 standard.

Iodine-starch test
Iodine-starch test is based on the reaction of iodine with starch and expressed by specific blue colouring. During comparison, only the coloured field sizes and proportions were evaluated, but not the colouration intensity or colour tone. The standard scale range is 10 points where 1 point – clear blue, apple ripening has not started, 10 points – the starch is broken up and the apple remains untangled (Lopez Camelo, 2004).

The following indices were calculated:
TSS/TA ratio;
Streif’s index (SI) F(R*S) (Streif, 1996);
De Jager’s index (PFW-1)F * (11 – S) / R (De Jager et al., 1996);
FARS index (F*A)/(R*S),

where: F – firmness, kg cm⁻²;
A – titratable acidity (TA), %,
R – soluble solids content (TSS), °Brix,
S – starch index (on a scale from 1 to 10).

Determination of ethylene production
Ethylene production rate was determined with the Ethylene analyzer ICA 56 based on quantity of ethylene formed by the fruits during the postharvest storage (Barker, 2002).

For ethylene determination one apple from each treatment was enclosed in about 4 L airtight jar for 24 h at 20 °C, and then ethylene production rates were measured. Ethylene production was given as µL h⁻¹ L⁻¹ kg⁻¹ (Oz, Ergun, 2009).
Sensory evaluation
The line scale method, based on the ISO 4121:2003 (Sensory analysis – Guidelines for the use of quantitative response scales) was used for this study. The samples were evaluated for taste, aroma, acidity, sweetness, juiciness, and colour change.

Statistical analysis
Data was processed by SPSS software version 17.0. Data was analysed using descriptive statistics and processed by one-way analysis of variance ANOVA (one way ANOVA), as well as for comparing all apple samples depending from the storage of type two-way analysis of variance ANOVA were used (two-way ANOVA). Microsoft Excel software version 2007 was used to determine significant differences between the samples.

Results and Discussion
The most important aspect in consumer’s choice of fruits and further inclusion in diet is sensory evaluation. The high quality apples were chosen to estimate fruit quality both after six months in different types of storage as well as after the shelves storage (shelf-life).

Sensory evaluation
Dramatic changes in apples quality have occurred during the storage in cooling chamber. From the seven of apple cultivars were retained only four of them. Analyzing data from sensory evaluation of apple colour (Figure 1a), which were stored in cooling chamber it was detected that highest assessment was for cultivar ‘Sinap Orlovskij’, while the lowest for ‘Auksis’ and ‘Zarja Alatau’. Statistical evaluation of colour data showed that between the samples there were no significant differences (p>0.05). Similar results were obtained during the evaluation of aroma changes in apple samples. The highest intensity of aroma was recorded for cultivar ‘Auksis’, which corresponds to 6.87 points. On the other hand the lowest intensity of aroma was observed for cultivar ‘Sinap Orlovskij’, which corresponds to 4.21 points. Comparing the data statistically it is seen that there were significant differences (p<0.05).

Comparing the data obtained after sensory evaluation of taste the intensity had the greatest reduction similarly like previously in ‘Sinap Orlovskij’, which conform 4.32 points. On the other side the highest intensity of taste was found in ‘Zarja Alatau’ and this amount corresponds to 7.89 points. Analyzing data obtained after sensory evaluation of apple acidity, experts were concluded that the intensity of acidity of cultivar ‘Sinap Orlovskij’ was not expressed so well (3.09 points). Comparing the data of the sweetness of apples there were no significant differences between the cultivars. Evaluation of apple juiciness showed that maximal value was recorded for cultivar ‘Belorusskoje Malinovoie’ (9.46 points), while the minimal value was for cultivar ‘Auksis’ (4.82 points). Comparing the data of apples after six months of storage, which had been stored in cooling chamber as well as after storage on shelves (shelf-life) it was found that the colour of apples after shelf-life period became more intensive for all cultivars. On the other side analyzing the data of apple sweetness was recorded that it’s became more intensive for cultivar ‘Belorusskoje Malinovoie’, while other attributes became less intensive (Figure 1b).

Obtained results suggest that apple storage in cooling chamber combination with 1-MCP treatment gives the possibility maintain and prolong the fruit expiration date till six months. Analyzing the obtained data after sensory evaluation (Figure 2a), it was found that the attributes like colour and aroma got significantly (p<0.05) lower assessments, while taste and juiciness has been more intensive comparing with control samples. The effectiveness of 1-MCP in decreasing the impact of aging on fruit physiology has been shown in a number of studies (Abdi et al., 1998; Golding et al., 1998; Fan, Mattheis 1999; Lurie et al., 2002; Defilippi et al., 2004; Kondo et al., 2005; Mattheis et al., 2005). On the other hand studies from Latvia (Juhnveica et al., 2013; Juhnveica-Radenkova et al., 2013) imply that 1-MCP treatment had a positive effect on apple sensory attributes.

Scientific studies from Michman and colleagues suggest that one of the most important negative aspects of 1-MCP is that, by eliminating sensitivity to ethylene, the production of volatile compounds is also reduced. These compounds contribute significantly to apple flavour and aroma (Michman et al., 2001). Our results coincide with the conclusions before, and unfortunately they are not recovered or partially recovering after two weeks of shelf-life (Figure 2b).

During the storage in ULO type cameras the colour and aroma of the samples (Figure 3a and Figure 4a) were partially developed. Statistically comparing the data it's seen that there were no significant differences between the ULO cameras (p<0.05). During the storage in ULO 1 camera it was observed one exception for cultivar ‘Gita’. During the storage of this cultivar intensive weight loss was occurred, hence this cultivar had not appropriate quality.
The intensive weight loss can be explainable by the fact that this cultivar was harvested too early. Comparing the data of sensory attributes (taste, acidity and juiciness) it is seen that there were no significant differences between the cultivars in ULO cameras (ULO 1 and ULO 2).

The results of this study showed that colour became more intense in all cultivars, while aroma was recovered in one part of cultivars (ULO 1: 'Orlik', 'Beloruskoje Malinovoje') and (ULO 2: 'Beloruskoje Malinovoje' and 'Zarja Alatau') (Figure 3b and Figure 4b). The evaluations of attributes like taste, acidity, sweetness and juiciness almost for all cultivars were much higher than for control samples and control, which was treated with 1-MCP. Obtained results can be explainable by the fact that during the storage in modified atmosphere decreased level of O$_2$ and increased level of CO$_2$ has a retarding effect on enzymatic activity and on morphological changes at a cellular level i.e. respiratory intensity, breakdown of membrane phospholipids and decrease of the volume of cytoplasm and mitochondria are all inhibited (Herregods, 1999).

### Apple firmness

In connection with reports from Poland scientists (Rutkowski et al., 2008) and scientists from Latvia (Juhecnica et al., 2009) another important indicator of fruit quality is tissue firmness, which play an important role in fruits choosing by consumers. The softening and cell separation in many fruits is associated with the increased proportion of readily soluble pectins. There are stages of cell wall breakdown during the ripening of apples. In the first stage fruit firmness declines slowly and wall galactans decrease. In the second stage, firmness decreases more rapidly and soluble polyuronides increase (Herregods, 1999). Reduction of the oxygen concentration to O$_2$ 2%, give the possibility significantly delay the second stage of fruit ripening (Knee, 1974). At the moment the standards for estimating the firmness of apple tissue, which characterize the quality of these fruits does not exist in Latvia. Based on the scientist from Poland (Rutkowski) and his unpublished works, apples can be considered like qualitative, when the firmness of the tissue not lower than 4.0 kg cm$^{-2}$, while the another scientist from America submits that firmness of qualitative apple should be not lower than 4.4 kg cm$^{-2}$ (Kupferman, 1992).

Taken into account the works from Poland scientists as well as after estimating the obtained data it is seen that our obtained results partially similar with Polish standards. Obtained results from ULO 1 type camera (Figure 5) showed that firmness of cultivar 'Auksis' is (4.7 kg cm$^{-2}$) and 'Sinap Orlovskij' (4.6 kg cm$^{-2}$). Comparing the data obtained in ULO 2 type camera practically all cultivars are comply with the Polish standards, except of cultivar 'Orlik' (2.9 kg cm$^{-2}$). Although after two weeks of apple storage on shelves (shelf-life) comparing with after six months of storage there were no found significant differences (p>0.05) between ULO cameras, and these results were no complying with Polish standards.

---

**FOODBALT 2014**

---

---
Detected only one exception for cultivar ‘Auksis’, which was stored in ULO 1 and the firmness corresponds with the standards (4.0 kg cm\(^{-2}\)).

**Conclusions**

Obtained results indicate that fruits, which were stored in modified atmosphere, as well as were treated with 1-MCP have less intensive aroma, flavour and colour. One of the possibilities how recover partially these attributes by storing the fruits for a short time (two weeks) on shelves (shelf-life).

Taken into account the evaluation of apple quality was recorded that apples which were stored in ULO type cameras have a distinctive juiciness and fresh taste. After six months of apple storage was detected that more appropriate storage for maintaining of apple firmness was ULO 2 type camera. All of these results comply with quality standards. On the other hand after two weeks of apple storage only one cultivar ‘Auksis’ (ULO 1 type) showed a positive value of firmness, while the other cultivars are not complying with the quality standards.

**Acknowledgment**

This research has been prepared within the State Research Programme ‘Sustainable use of local resources (earth, food, and transport) – new products and technologies (NatRes)’ (2010–2014) Project no. 3. ‘Sustainable use of local agricultural resources for development of high nutritive value food products (Food)’.

**References**


\[\text{Figure 5. Firmness, kg cm}^{-2}\text{, depending on storage conditions}\]

After six months of storage (shelf-life).

- 'Orlik'
- 'Belorusiskoje Malinovoje'
- 'Ortik'
- 'Gita'
- ‘Auksis’

©FOODBALT 2014
transport) – new products and technologies (NatRes).

B. Andersons, V. Šegliņš, D. Dubrovski, R. Galoburda, A. Paeglītis (eds.). Riga, Latvia, p. 207–211. (In Latvian)


Cranberries belong to a group of evergreen dwarf shrubs or trailing vines in the genus *Vaccinium* subgenus *Oxyccocos*. Berries contain a diverse array of nutrients with recognized biological activities that promote or contribute to health. The aim of the research was to evaluate physically-chemical parameters of Latvian fresh cranberries. The research was accomplished on fresh Latvian wild and large-berry cranberries harvested in Kurzeme region: wild cranberries and large-berry cranberries variety ‘Steven’, ‘Ben Lear’, ‘Bergman’, ‘Pilgrim’ and ‘Early Black’. The following quality parameters of cranberries were analysed using standard methods: anthocyanins (spectrophotometric), colour (using the colour system CIE L\*a\*b\*), organic acids (high-performance liquid chromatography (HPLC)), polyphenols (HPLC), and pH value (LVS ISO 1132:2001). The research results confirm a close interaction (strong positive correlation (r=0.919) between the colour a\* component intensity and anthocyanin content. The highest content of benzoic acid was determined in large-berry cranberries of the cultivar ‘Early Black’ – 13.66 mg 100 g\(^{-1}\), which was by 56% higher than in wild cranberries – 5.98 mg 100 g\(^{-1}\), and by 65% higher than in the cultivar ‘Steven’ berries – 4.82 mg 100 g\(^{-1}\). The highest content of polyphenol compounds was found in cranberries of the cultivars ‘Pilgrim’, ‘Early Black’, and ‘Steven’, while the lowest – in cranberries of the cultivar ‘Bergman’. The average pH value for all samples was 2.465; it is slightly lower than found in the scientific literature.

**Keywords**: anthocyanins, organic acids, polyphenols, cranberries.

**Introduction**

Cranberries are extremely versatile berries. When incorporated into other food products, they provide refreshing flavour as well as a characteristic red colour. Used in combination with other fruits and berries, cranberries can accentuate and enhance the flavours of these fruits and berries. Because of their health gains, cranberries are experiencing an expansion into the food and beverage industry. Available year-round and in a variety of forms, cranberries can be used to improve numerous products and applications in the food and beverage industry (Hui et al., 2006).

Anthocyanins (of the Greek *anthos* = flower and *kianos* = blue) are the most important pigments of the vascular plants; they are harmless and of easy incorporation in aqueous media, which makes them interesting for its use as natural water-soluble colorants (Pazmiño-Durán et al., 2001). Anthocyanins are glycosylated polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium (flavylium) salt which are natural colorants widely distributed in nature (Bordignon-Luiz et al., 2007; Hendry, Houghton 1996). Many researches indicate that anthocyanins are not only nontoxic and nonmutagenic, but they have positive therapeutic properties such as antioxidant, anti-inflammatory, anticarcinogenic, antiviral, and antibacterial properties (Tall et al., 2004).

A limiting factor for the incorporation of anthocyanins in foods is the low stability of these pigments which are influenced by several factors: most important the pH of a system. They are susceptible to light and temperature, different agents can also cause their degradation (oxygen, enzymes etc.) (Jackman, Smith, 1996). The content of anthocyanins in cranberries equals to 20–360 mg 100 g\(^{-1}\) (Wang, Stretch, 2001; Prior et al., 2001). Colour is an important factor in the consumer’s choice of food products. It is one of the most important characteristics used to define the quality of food and has a decisive influence on the acceptance by the consumer (Hendry et al., 1996). CIELab is a nonlinear transformation of XYZ into coordinates L\*, a\*, b\* and it is used for the colour measurement interpretation (Hoffmann, 2008). Organic acids are primary metabolites, which can be found in great amounts in all plants, especially in berries. Organic acids are naturally found in fruits and berries. Many berries contain a variety of free acids which are colourless compounds soluble in water, ethanol; but insoluble in the nonpolar solvents benzene or petroleum ether. These acids are weakly acidic and can accumulate in the cellular vacuoles of plants. Some of the acids are components of the citric acid cycle, while others are intermediates in the pathway leading from carbohydrates to either aromatic compounds or isoprenoid derivatives (Arslan, Özcan, 2011; Dashek, Micales, 1997). Organic acids are biologically important because they form part of different biochemical metabolic routes such as the Krebs cycle (Arslan, Özcan, 2011; Silva et al., 1991). The concentration of organic acids is an important factor influencing the organoleptic properties of berries (Koyuncu, Dilmaçūnl, 2010; Lee, 1993).

Phenolic compounds are secondary metabolites that are derivatives of the pentose phosphate and phenylpropanoid pathway in plants. These compounds one of the most widely occurring groups of phytochemicals are of considerable physiological and morphological importance in plants (Puppone-Pimia, 2001). Many small fruit phenolic compounds are good sources of natural antioxidants and have inhibitory effects on mutagenesis and carcinogenesis. During the past decades, extensive analytical research has been carried out on the separation and determination of...
phenolic constituents in various fresh fruit products and environmental samples. The unique antibacterial activities of cranberry implicate that cranberry may possess a very different flavonoid and phenolic composition from other kinds of fruits. An efficient separation and quantitation method is essential for understanding the components of flavonoid and phenolic antioxidants in cranberry and their health benefits. Phenolic compounds are not temperature resistant; therefore, treatment at elevated temperatures will negatively influence polyphenol compound activity (Chen et al., 2001). Therefore, the aim of the current research was to evaluate physically-chemical parameters of Latvian fresh cranberries.

Materials and Methods
The research was accomplished on fresh Latvian wild and large-berry cranberries harvested in Kurzeme region in 2010: wild cranberries and large-berry cranberries variety 'Steven', 'Ben Lear', 'Bergman', 'Pilgrim' and 'Early Black'. During the experiments, the following quality parameters of cranberries were controlled using standard methods:

- anthocyanins were determined by means of "Spectrophotometer Anthocyanins Determination Method" using a 6705 UV/VIS Spectrophotometer JENWAY (Bordignon-Luiz et al., 2007);
- a colour parameters of fresh cranberries were determined by direct reading with a COLOR TEC PMC. The colour parameters such as luminance (L*) ranging from 0 (black) to 100 (white) on a vertical axis, red saturation index (a*), and yellow saturation index (b*), were evaluated (McGuire, 1992);
- pH of berries was measured using potentiometric method LVS ISO 1132:2001 using Jenway 3510 pH metre. Prior to the determination of pH, cranberries were chopped by the blender; organic acids were extracted with water using the method of Hernandez et al. (2009);
- phenolic compounds were determined using a high-performance liquid chromatography (HPLC) with UV detection (at 280 nm) (Berregi et al., 2003).

Microsoft Excel software was used for the research purpose to calculate mean arithmetical values and standard deviations of the mathematical data used in the research. SPSS 20.0 software was used to determine the significance of research results, which were analysed using the following test methods: Sheffe test, two-factor ANOVA, and three-factor ANOVA analyses to explore the impact of factors and their interaction, and the significance effect (p-value).

Results and Discussion

Anthocyanins
The anthocyanins give to cranberries bright red colour and work like antioxidant. The changes of temperature during berries growing are closely related with low content of anthocyanins in the cranberries. For example, the high temperature inhibits the formation of anthocyanins in the berries (Prior et al., 2001). During present research it was detected that the highest anthocyanin content was in large-berry cranberries variety 'Pilgrim' and 'Early Black', respectively 898±12 mg 100 g⁻¹ and 839±8 mg 100 g⁻¹ in dry matter although what was by 66% and 63% higher comparing to anthocyanin content in wild cranberries. The lowest anthocyanin content was detected in wild cranberries, as 306±4 mg 100 g⁻¹ (Figure 1), and in large-berry cranberries variety 'Ben Lear' – 492±4 mg 100 g⁻¹.

Obtained results suggest that in the large-berry cranberries varieties 'Pilgrim' and 'Early Black' is the lowest red pigment intensity – they are not so red.

In scientific literature is mentioned, that the anthocyanin content in Latvian large cranberries is in the range from 186 mg% to 604 mg% (Ripa, 1992). Different results were obtained by Wang, Stretch (2001) and Prior et al., (2001), where anthocyanin content in cranberries is in the range from 360 mg 100 g⁻¹. Such differences could be explained with the berry cultivar and the characteristics of growing conditions.

Intensity of colour compounds
The L* colour compound value indicates colour saturation level in the product. The research data show, that the colour of wild cranberries (23.39±1.22) is darker than colour of large-berry cranberries (Figure 2). If compare the large-berry cranberries 'Steven' and wild cranberries, the 'Steven' berries are brighter – L* colour compound value is 17.00±0.69.

The a* colour compound value mainly indicate the red colour of product. As results of the current research demonstrate, cranberry of variety 'Pilgrim' are redder...
(a* colour compound value is 15.28±0.74) (Figure 3). The 'Pilgrim' berries red colour is on average by 40% higher comparing to wild cranberries – the red colour value was 9.15±0.69. Red colour intensity changes are closely related with the anthocyanins content in the berries. It is the reason, why large cranberries have more pronounced colours (Figure 1), what mainly could be explained with higher content of anthocyanins in berries.

The research results confirm a good correlation (r=0.919) (Figure 3) between the colour a* component intensity and anthocyanin content in analysed cranberries.

**pH**

Significant difference in pH value was detected between cranberries varieties 'Steven' and 'Pilgrim' (p=0.020; α=0.050), and between 'Steven' and 'Early Black' (p=0.020; α=0.050) (Figure 4). The average pH value for all berries samples was 2.465; it is slightly lower than found in the scientific literature – the pH value of cranberry juice is 3.000 (Ripa, 1992).

Possible presence and abundance of organic acids, mainly citric, malic, quinic, and benzoic acids, are responsible for cranberries characteristically low pH (Figure 4). These acids also aid in the stabilization and protection of anthocyanins, and help protect the much desired red color of cranberries. In theory, the pH should also affect the cranberry phenolic ability to inhibit lipid peroxidation and their free radical-scavenging capacity (Cailleta et al., 2011).

**Organic acids**

Cranberries are rich in bioactive components, such as, phenols and organic acids, which can provide berry antimicrobial activity (Puupponen-Pimia et al., 2001; Rauha et al., 2000).

Organic acids were determined in cranberries as follow: tartaric acid, quinic acid, malic acid, succinic acid, benzoic acid (Fig. 5).

It should be noted that the benzoic acid content is one of the most important parameters for berry bactericidal properties. The highest content of benzoic acid was determined in large-berry cranberries of the variety 'Early Black' – 13.66 mg 100 g⁻¹, which was by 56% higher than in wild cranberries – 5.98 mg 100 g⁻¹, and by 56% higher than in the variety 'Steven' berries – 4.82 mg 100 g⁻¹ (Figure 5). The mathematical data processing outlines that the benzoic acid content is significantly different in all cranberry varieties (p=0.0001; α=0.0500), except wild cranberries and cranberries of the variety 'Ben Lear' (p=0.0430; α=0.0500). There are no significant differences between the large-berry variety 'Steven' and 'Ben Lear' (p=0.0500; α=0.0500) cranberries.

The highest salicylic acid content was found in cranberry variety 'Pilgrim' – 0.2434±0.0032 mg 100 g⁻¹ it was by 15% higher comparing to salicylic acid content in wild cranberries – 0.2066±0.0062 mg 100 g⁻¹ and by 60% higher comparing to salicylic acid content in 'Bergman' cranberries – 0.0968±0.0105 mg 100 g⁻¹ in dry matter (Figure 6). In the present research it was proved, that in cranberries is highest hinic acid content (24.0000–29.0000 mg 100 g⁻¹) (Figure 5), however lowest (Figure 6) – salicylic acid content (0.0968–0.2434 mg 100 g⁻¹). Such difference mainly could be explained with individuality of the variety (Celik et al., 2008).

**Polyphenols**

The most important polyphenol function is protection of plant decay modes (Dixon, Paiva, 1995; Bennet, Wallsgrove, 1994). There are caused by phenol
changes, like excessive exposure to UV light, mechanical damage or microbial infection. The environmental factors have the significant effect on the content of phenolic compounds in berries. Within present research it was established that in cranberry more popular polyphenols are rutin, catechin, caffeic acid and chlorogenic acid (Figure 7).

**Figure 7. Content of polyphenols in fresh cranberries (in dry matter)**


The highest content of analysed compounds was found in cranberries of the variety 'Pilgrim', 'Early Black', and 'Steven'; while the lowest – in cranberries of the variety 'Bergman' (Figure 7). There is a significant difference (p<0.0001, a=0.0500) in the content of polyphenol in cranberries.

**Conclusions**

The highest anthocyanin content was found in cranberry varieties 'Pilgrim' and 'Early Black', the lowest in wild cranberries. The colour of wild cranberries is darker then the colour of large-berry cranberries. The highest content of benzoic acid was determined in large-bery cranberries of the variety 'Early Black', which was by 56% higher than in wild cranberries – 5.98 mg 100 g⁻¹, and by 56% higher than in the variety 'Steven' berries – 4.82 mg 100 g⁻¹ in dry matter. The highest salicylic acid content was found in cranberry variety 'Pilgrim' berries; it was by 15% higher comparing to salicylic acid content in wild cranberries and by 60% higher comparing to salicylic acid content in 'Bergman' cranberries. The highest content of polyphenol compounds was found in cranberries of the variety 'Pilgrim', 'Early Black', and 'Steven'; while the lowest – in cranberries of the variety 'Bergman'.

**Acknowledgment**

The research and publication has been prepared within the framework of the ESF Project “Formation of the Research Group in Food Science”, Contract No. 2009/0232/1DP/1.1.1.2.0/09/APIA/V1A/122.

The research and publication has been prepared within the framework of the ESF Project “Support for LLU doctoral studies implementation”, Contract No. 2009/0180/1DP/1.1.1.2.0/09/APIA/V1A/017.

The authors would like to express acknowledgements to Fredijs Dimins from Latvia University of Agriculture, Department of Chemistry, for technical advice in organic acids and polyphenol determination.

**References**


PHYSICAL AND CHEMICAL PARAMETERS OF STRAWBERRY PUREE

Ruta Galoburda1, Sigit Boca2, Imants Skrupskis2, Dalija Seglina3

1 Department of Food Technology, Faculty of Food Technology, Latvia University of Agriculture, Liela Street 2, Jelgava, Latvia, e-mail: ruta.galoburda@llu.lv
2 Department of Nutrition, Faculty of Food Technology, Latvia University of Agriculture, Liela Street 2, Jelgava, Latvia
3 Latvia State Institute of Fruit Growing, Graudu Street 1, Dobele, Latvia

Abstract

Practice of the world shows, that more and more often a consumer chooses products, which can be used in nutrition without spending much time for their preparation. Use of fruit desserts in nutrition is one of the ways how to consume vitamins and minerals. The aim was to investigate physical and chemical parameters of strawberry puree from different cultivars and the effect of freezing on its quality. The strawberry cultivars - ‘Polka’, ‘Honeoye’, and ‘Senga Sengana’ harvested in Latvia were used in the study. The strawberries were processed in a blender until obtaining a homogenous mass. The strawberry mass was analyzed fresh and after storage at –18 °C. Content of soluble solids was determined according to ISO 2173:2003 using digital refractometer; content of total phenols by spectrophotometer method. Content of total acids was explicit as citric acid. Content of sugars (sucrose, glucose, and fructose) was evaluated by high performance liquid chromatography (HPLC). Content of anthocyanins was evaluated by spectrophotometer method. pH was measured according to LVS 1132:2001, content of vitamin C - AOAC Official Method 967.21. Colour was measured in CIE L*a*b* system. Content of vitamin C in strawberry puree essentially differs (p<0.01) among the researched cultivars and among the types of thermal treatment. Content of vitamin C in strawberry puree has a close correlation with content of total sugars. Close correlation of a* (r=0.810) and b* (r=0.851) with content of total sugars was established. This could be explained with the changes of the content of anthocyanins in strawberry puree. Content of total acids in fresh strawberry puree from the researched cultivars was from 0.85±0.03 to 0.87±0.03 mg 100 g–1, which increases in average for 4.4% as a result of freezing. The highest content of anthocyanins was in fresh strawberry puree from the cultivar ‘Polka’, whereas the lowest – from the cultivar ‘Honeoye’.

Keywords: strawberry puree, freezing, anthocyanins, sugars, vitamin C, colour.

Introduction

Practice of the world shows, that more and more often a consumer chooses products, which can be used in nutrition without spending much time for their preparation. Use of fruit desserts in nutrition is one of the ways how to consume vitamins and minerals. Strawberries contain 80–90% water, 0.9–1.2% fibre, 4.5–10% sugar, 0.17–0.25% tannins, vitamins B1, B2, K, carotene, folic acid, iron, potassium, calcium (Blanda et al., 2009). Strawberries are known as a good source of vitamin C, folicates (folic acid) and recently also as a product with high content of various phenols (Proteggente et al., 2002), majority of which exhibits antioxidative capacity (Tulipani et al., 2011). Strawberry quality is also described by content of minerals, carbohydrates, organic acids – malic acid, tartaric acid, citric acid. Citric acid forms around 90% of organic acids present in strawberries (Sturm et al., 2003). It is reduced along ripening and depends on the cultivar (Brasileiro et al., 2011).

According to Sturm et al. (2003) and Pelayo et al. (2003) the most important strawberry quality indicators are content of sugars and total acids, which can be described as a sugar / acid ratio (Pineli et al., 2011). Sugar / acid ratio characterizes degree of sweetness, which depends on cultivar, ripeness, and weather conditions. Sturm et al. (2003) in their research established different results, where total content of acids in some cultivars increased during fruit ripening (content of citric acid in strawberries of cultivar ‘Miss’ increased from 0.40 g 100 g–1 till 1.00 g 100 g–1). This study indicated presence of tartaric acid in amount of 0.10±0.04 g 100 g–1. The content of sucrose and glucose was the smallest among all sugars detected in studied strawberries, whereas fructose content was the highest (Sturm et al., 2003). According to the study of Pelayo et al. (2003) dry matter in strawberries was 7.8–16.6%; total sugars (fructose, glucose, sucrose) were 4.4–10.8%, organic acids – 0.49–1.61%. Pineli et al. (2011) observed that values of total anthocyanins increased approximately 9–13-fold from green to pink stage. Cyanindin-3-glucoside contributes to a red colour, whereas pelargonidin is a red-orange pigment. Strawberries are one of the most widely consumed fruits both in fresh and processed form – such as juice, beverage, jam, puree or powder. Storage of fresh strawberries is complicated because they are perishable soft fruits exhibiting an extremely short postharvest shelf-life due to high content of water. Freezing is the best and most effective method of fruit preservation, which maintains taste, appearance, flavour and nutritional value of fresh fruits (Sturm et al., 2003; Ancos et al., 2000; Skrede, 1996). More and more often fruit is frozen for further processing and it is important to study effect of freezing on quality of final products (Skrupskis et al., 2011; Kampuse et al., 2005). Only few researches are available on freezing of fruit puree (Huang et al., 2013). Researchers concluded that fruit with high content of soluble solids are less suitable for freezing (Modise, 2008; Kampuse et al., 2003). Sour fruit juice (Boca et al., 2011), sugar, sugar syrup or honey can be used for puree colour stabilisation during its storage and thawing. Quality of fruit puree is influenced by many factors – differences in cultivars, changes of physical and chemical and

172
textural parameters during thermal treatment and storage, sensory parameters, as well as microbiological quality.

The aim of the current research was to investigate physical and chemical parameters of strawberry puree from different cultivars and the effect of freezing on its quality.

Materials and Methods

Materials

Strawberries of the cultivars ‘Polka’, ‘Honeoye’, and ‘Senga Sengana’ at full ripeness stage, harvested at the end of June or at the beginning of July depending on the type of cultivar, were used for the study. After harvesting, the strawberries were sorted, cleaned, washed, processed in a HR 2000/70 blender (Philips, China) until obtaining a homogenous mass, and placed into 200 mL plastic vessels. The prepared mass was analysed – fresh (just after preparation) and frozen (frozen at -25±2 °C for 20 h, stored in a frozen condition at -18±2 °C for three months, thawed for 16 h till the product temperature reached +4±2 °C; further in the text – frozen puree).

Methods

For measurement of soluble solids content, an ATAGO N20 digital refractometer (Atago Co., Ltd, Japan) was used according to ISO 2173:2003.

pH was determined using a Jenway pH meter 3510 (Jenway, UK) according to the standard LVS EN 1132 **“pH Determination of Fruit and Vegetable Juice”**.

Content of vitamin C was determined by 2,6-dichloroindophenol titrimetric method (AOAC Official Method 967.21).

Content of sugars (sucrose, glucose, and fructose) was determined using a LC-10AD Prominence (Shimadzu Japan) high performance liquid chromatograph (HPLC) equipped with refractive index detector RID-10A and autosampler SIL-10AF. Test conditions: mobile phase mixture of acetonitrile with deionized water in ratio 80 : 20 (v/v); flow rate – 0.6 mL min⁻¹; temperature of column and detector – +50 °C; volume of the injected sample – 10 µL.

Strawberry puree samples for sugar testing were prepared adding 40 mL of deionized water (+80 °C) to 10 grams of puree and mixing it for 1 min. Then sample was centrifuged (10 000 g / 5 min at +20 °C). The procedure was repeated twice, then supernatant was cleared, and finally filtered through membrane filter (0.45 µm). Each sample was analysed in triplicate. Chromatograph software calculated content of glucose, fructose, and sucrose comparing chromatograms of puree with standard curves of respective sugar.

Content of total acids was determined by potentiometric titration method and was converted to citric acid.

The total phenolic content (TPC) of strawberry puree was determined according to the Folin-Ciocalteu spectrophotometric method (Singleton et al., 1999) with some modifications. For analysis 4–6 grams of puree was taken. The absorbance was measured at 765 nm using a UV–1650 PC spectrophotometer (Shimadzu, Japan) twice for each of the duplicate samples. Total phenols were expressed as gallic acid equivalents (GAE) per 100 grams of puree.

The content of anthocyanins was determined by the spectrophotometric method (Moor et al., 2005). For determination, 20 grams of strawberry puree was homogenized together with 40 g of ethanol and hydrochloric acid mixture (85 : 15 v/v) for 1 min. After filtration sediments were rinsed three times with 10 mL ethanol and 1.5 M HCl reagent (3 x 10 mL). The absorbance of filtrate was measured at 535 nm using a UV-1650 PC spectrophotometer (Shimadzu, Japan) in triplicate.

Colour was measured in CIE L*a*b* colour system by a colorimeter (Tec PCM/PSM, USA). Strawberry puree before colour measurement was placed in a 100 mL PET containers, to have the same thickness of puree layer. Colour was measured in eight randomly selected spots. Data was processed in ColorSoft software.

A colour spectrum was determined in a three coordinate system: L* represents lightness, where L* =100 – white, L* =0 – black. Value of colour component a* is from -a* (green) to +a* (red), component b* – from -b* (blue) to +b* (yellow). Colour changes in puree after freezing compared to fresh puree were described by total colour difference ΔE* (MacDougall et al., 2002). Total colour difference (ΔE*) was calculated according to the equation:

\[
\Delta E^* = \sqrt{(L^* - L^*_0)^2 + (a^* - a^*_0)^2 + (b^* - b^*_0)^2},
\]

where \( L^*_0; a^*_0; b^*_0 \) – values for fresh sample; \( L^*; a^*; b^* \) – values for frozen sample.

In the research calculations were carried using MS Excel program and SPSS 16 statistics software. The hypothesis set was checked with the p-value method. Factors were estimated as significant when the p-value was <α. For interpretation of the results it is assumed, that α=0.05 with 95% reliability, if not indicated otherwise. The following tests and analyses were used: one-factor and two-factor variance analysis (ANOVA), correlation and regression analysis. If the correlation between variables is linear, the determination coefficient coincides with the correlation coefficient: \( R^2 \) is equal to \( r^2 \). If the value of correlation coefficient is 0.5≤|r|≤0.8, then there is medium close linear correlation between the examined variables (Arhipova, Bāliņa, 2006).

Results and Discussion

Soluble solids

Soluble solids content in fresh strawberry puree samples ranged from 8.54 °Brix to 10.50 °Brix...
After freezing and thawing cycle content of soluble solids decreased possibly due to drip loss.

Table 1

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Soluble solids, °Brix</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>fresh</td>
</tr>
<tr>
<td>'Senga Sengana'</td>
<td>9.32±0.06</td>
</tr>
<tr>
<td>‘Polka’</td>
<td>10.50±0.08</td>
</tr>
<tr>
<td>‘Honeoye’</td>
<td>8.54±0.19</td>
</tr>
</tbody>
</table>

Pineli et al. (2011) observed lower content of soluble solids (5.0–7.9 °Brix).

Values found for pH tended to be lower for strawberries of cultivar ‘Honeoye’ (Table 2).

Table 2

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>fresh</td>
</tr>
<tr>
<td>'Senga Sengana'</td>
<td>3.43±0.02</td>
</tr>
<tr>
<td>‘Polka’</td>
<td>3.44±0.01</td>
</tr>
<tr>
<td>‘Honeoye’</td>
<td>3.24±0.02</td>
</tr>
</tbody>
</table>

pH determined for strawberry purees in the current research are within the same range as observed by Pineli et al. (2011).

Content of vitamin C

Content of vitamin C in strawberry puree essentially differed (p<0.01) among the researched cultivars (Table 3).

Table 3

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Content, mg 100 g⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>fresh</td>
</tr>
<tr>
<td>'Senga Sengana'</td>
<td>23.51±0.38</td>
</tr>
<tr>
<td>‘Polka’</td>
<td>21.13±0.23</td>
</tr>
<tr>
<td>‘Honeoye’</td>
<td>19.40±0.27</td>
</tr>
</tbody>
</table>

The most significant differences were found between puree made from ‘Senga Sengana’ and ‘Polka’, as well as puree of ‘Senga Sengana’ and ‘Honeoye’. Content of vitamin C in fresh puree was between 19.40±0.27 mg 100 g⁻¹ (‘Polka’) and 23.51±0.38 mg 100 g⁻¹ (‘Senga Sengana’). During fruit homogenization air is incorporated in puree, consequently content of vitamin C is reduced due to presence of oxygen. As a result fruit puree contains smaller amount of vitamin C compared to whole fruits. Literature data show that vitamin C content in strawberries (not puree) of cultivar ‘Polka’ was 58–63 mg 100 g⁻¹ (Laugale et al., 2000); Finnish researchers depending on harvest year detected its content from 48.0±1.6 to 62.0±1.6 mg 100 g⁻¹ (Hakala et al., 2003). Whereas strawberries of cultivar ‘Honeoye’ contained more vitamin C – 80–87 mg 100 g⁻¹ (Laugale et al., 2012) and 54.1±3.7–71.3±3.1 mg 100 g⁻¹ (Hakala et al., 2003). In cultivar ‘Senga Sengana’ it was from 43.6±2.5 to 54.2±2.5 mg 100 g⁻¹ (Hakala et al., 2003). Another Finnish study (Hägg et al., 1995) in various fresh strawberries determined 56–99 mg 100 g⁻¹ of vitamin C, which decreased by 34% as a result of freezing. Our research reveals that content of vitamin C is significantly higher (p<0.01) in fresh puree compared to puree after freezing. The decrease of vitamin C content in all studied purees was 18.8% on average. Velde et al. (2013) demonstrated that content of vitamin C in frozen strawberries was 44.5±3.2 mg 100 g⁻¹.

Vitamin C content has a close correlation with total sugar content in strawberry puree (r=0.830). Data shows: the higher total sugar content in strawberry puree, the higher vitamin C content (Fig. 1).

Figure 1. Correlation between content of vitamin C and total sugars in strawberry puree

Regression equation shows – if total sugar content is increased by 1 g 100 g⁻¹, content of vitamin C will increase by 1.56 mg 100 g⁻¹. It can be explained by the processes taking place during fruit maturation – both synthesis of vitamin C and sugars occurs simultaneously.

Content of total sugars

There is no essential difference (p=0.117) in content of total sugars of strawberry puree between cultivars, whereas it is essentially (p<0.001) influenced by the freezing (Fig. 2). Total sugar content in fresh strawberry puree is from 7.82 to 8.17 g 100 g⁻¹, which is similar to the result established for strawberry variety ‘Mohawk’ – 7.41 g 100 g⁻¹ (Sturm et al., 2003); whereas German study (Keutgen et al., 2007) show total sugar content of 6.33±0.39 g 100 g⁻¹, which is lower compared to our study. Total sugar content in strawberry puree decreased during freezing in average by 41.9%.

Content of individual sugars – sucrose, glucose and fructose in both fresh and frozen strawberry puree is presented in Figure 3.
however it is essentially (p<0.001) influenced by the freezing.

Content of total acids
Content of total acids in fresh strawberry puree does not essentially (p=0.164) differ among cultivars and it is from 0.85±0.03 to 0.87±0.03 mg 100 g⁻¹, which increases in average for 4.4% as a result of freezing (Table 4).

Table 4

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Content, mg 100 g⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>fresh</td>
</tr>
<tr>
<td>‘Senga Sengana’</td>
<td>0.86±0.03</td>
</tr>
<tr>
<td>‘Polka’</td>
<td>0.87±0.03</td>
</tr>
<tr>
<td>‘Honeoye’</td>
<td>0.85±0.03</td>
</tr>
</tbody>
</table>

Content of total acids has a negative close correlation with content of vitamin C in strawberry puree (r=0.820). Data show that content of vitamin C is lower in strawberry puree, which has higher content of total acids. This can be explained by the ripeness stage of strawberries. This is also confirmed by close negative correlation (r=0.916), which exists between content of total sugars and acids in strawberry puree (Figure 4).

The correlation shows that the increase of total sugars by 1 g 100 g⁻¹, results in decrease of total acids in strawberry puree by 0.0169 g 100 g⁻¹. This might be due to the physiological properties of fruits when with increased stage of ripeness increase in sugar content and decrease in total acids is observed (Brasileiro et al., 2011). Sugar/acid ratio was not significantly different among cultivars (p=0.138) and it ranged from 9.2 to 9.4. Fresh strawberry purees from researched cultivars may be characterized as “sour”, which sugar/acid ratio does not change essentially during freezing – they also can be characterised as “sour”. Sturm et al. (2003) found sugar/acid ratio at full maturity stage around 6.5 in average, with higher values for some cultivars – 8.7 (cultivar ‘Mohawka’), whereas Keutgen et al. (2007) reported sugar/acid ratio around 10.13, which is similar to our result.
Content of total phenolic compounds

Content of total phenolic compounds in strawberry puree (Table 5) does not significantly differ among cultivars (p=0.471).

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Content, mg 100 g⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>fresh</td>
</tr>
<tr>
<td>'Senga Sengana'</td>
<td>141±14</td>
</tr>
<tr>
<td>'Polka'</td>
<td>181±16</td>
</tr>
<tr>
<td>'Honeoye'</td>
<td>130±11</td>
</tr>
</tbody>
</table>

Table 5

Content of total phenols in strawberry puree

Pineli et al. (2011) reported higher content of phenols in fresh strawberries compared to our results 223.46±6.96 mg 100 g⁻¹. Our research revealed that content of total phenols in fresh strawberry puree essentially differs (p=0.001) from their content in frozen puree – in average by 91%. The same conclusion was drawn in research of Blanda et al. (2009), who established higher total phenol content in frozen fruits compared to fresh ones. Higher content possibly was formed in hydrolysis of polymers or in metabolism processes, which is created by osmotic stress in fruits (Blanda et al., 2009).

Content of anthocyanins

Content of anthocyanins in strawberry puree was essentially (p<0.01) different among the researched cultivars (Figure 5). The highest content of anthocyanins was in fresh strawberry puree from the cultivar ‘Polka’ (30.04±0.63 mg 100 g⁻¹), whereas the lowest – from the cultivar ‘Honeoye’ (14.91±0.41 mg 100 g⁻¹).

Download Table 5

Figure 5. Content of anthocyanins in strawberry puree

During freezing their content decreased for 2.05% to 11.32%. During freezing content of anthocyanins was preserved better than under the influence of pasteurisation, alongside better preserving the red colour characteristic to strawberries (Boca et al., 2011). Content of anthocyanins has a close correlation with the content of soluble solids in strawberry purees (r=0.833). In strawberry purees, with higher content of soluble solids, the content of anthocyanins was higher.

Literature show anthocyanins content in strawberries 10–80 mg 100 g⁻¹. Pineli et al. (2011) indicated that anthocyanins content in unripe berries was 2.35±0.35 mg 100 g⁻¹, and it increased till 22.64±0.66 mg 100 g⁻¹ upon ripening. Aaby et al. (2012) study demonstrated higher anthocyanins content, in fresh strawberries of cultivar ‘Polka’ it was 37.2 mg 100 g⁻¹, in ‘Senga Sengana’ – 27.4 mg 100 g⁻¹. Whereas Velde et al. (2013) in frozen strawberries established similar anthocyanins content to our study 22.1±4.2 mg 100 g⁻¹.

Strawberry puree colour

Colour component a*, b* and L* values of fresh and frozen strawberry puree does not differ essentially in cultivars (p>0.05), whereas there are essential (p<0.001) differences of them in the types of treatment. This is also shown by total colour changes (Fig. 6).

Figure 6. The influence of freezing on the total colour difference (ΔE*) of strawberry puree

Colour component a* and b* value of strawberry puree has a close correlation with content of total sugars in strawberry puree. Data, displaying the close correlation a* (r=0.810) and b* (r=0.851) for the value with content of total sugars in strawberry puree, show that in samples, where content of total sugars is higher, red colour (a*) and yellow (b*) colour intensity increases. This could be explained with the changes of the content of anthocyanins in strawberry puree, because they form red colour characteristic to strawberries and they are glucosidically bound with sugar.

Conclusions

Content of vitamin C in strawberry puree essentially differs (p<0.01) among the researched cultivars and between fresh and frozen-thawed puree. Close correlation of colour components a* (r=0.810) and b* (r=0.851) with content of total sugars was established. Content of total acids in fresh strawberry puree from the researched cultivars was from 0.85±0.03 to 0.87±0.03 mg 100 g⁻¹, which increases in average by 4.4% as a result of freezing. The highest content of anthocyanins was in fresh strawberry puree from the cultivar ‘Polka’, whereas the lowest – from the cultivar ‘Honeoye’.

References

INFLUENCE OF OENOCCOCUS OENI AND OAK CHIPS ON THE CHEMICAL COMPOSITION AND SENSORY PROPERTIES OF CIDER

Rita Riekstina-Dolge1, Zanda Kruma1, Ingmars Cinkmanis2, Evita Straumite1, Martins Sabovics1, Lolita Tomson2

1 Department of Food Technology, Faculty of Food Technology, Latvia University of Agriculture, Liela street 2, Jelgava, Latvia, e-mail: ritard@llu.lv
2 Department of Chemistry, Faculty of Food Technology, Latvia University of Agriculture, Liela street 2, Jelgava, Latvia

Abstract

Cider quality is influenced by several factors, namely, apple variety, yeast strains, fermentation and maturation conditions. The aim of current research was to evaluate influence of lactic acid bacteria Oenoococcus oeni and oak chips on the quality of apple cider. After main fermentation lactic acid bacteria Oenococcus oeni (LAB) and two types of oak chips: unroasted oak chips (U_OC) and medium roasted oak chips (MR_OC) were added to cider and samples were matured for four weeks. For matured ciders total phenolic compounds (TPC) were determined spectrophotometrically, individual phenolic compounds by HPLC, volatile compounds by SPME followed by GC/MS and sensory properties using line scale and ranking test. The TPC in ciders ranged from 1028 mg L⁻¹ in cider LAB to 1526 mg L⁻¹ in cider MR_OC. Among analysed phenolic compounds chlorogenic acid dominated in all samples. In cider MR_OC comparing with control sample higher content of caffeic acid, epicatechin, ferulic acid and vanillin was determined. The highest total peak area was determined in cider U_OC. Principal component analysis showed that profile of volatile compounds can be explained by three factors; the first two represent 81% of the total variances. The characteristic volatiles of cider LAB were acetic acid, ethyl-9-decanoate, ethyldecanoate, octanoic acid, and ethylhexanoate, in cider MR_OC 3-methyl-1-butanol acetate, butyl acetate and ethyl acetate, whereas in cider U_OC 3-methyl-1-butanol and phenylalcohol. Preference ranking test results showed that the assessors preferred cider LAB. Addition of Oenococcus oeni, unroasted and roasted chips during maturation significantly influence chemical composition and sensory properties intensity of cider.

Keywords: cider, maturation, Oenococcus oeni, oak chips

Introduction

The quality of fermented drinks like cider depends on presence of aroma compounds in product, that are influenced by several factors, namely apple variety, yeast strains, fermentation conditions, the production process and fining treatments (Hidalgo et al., 2004; Martínez-Rodriguez, Polo, 2003). Quality of ciders could be improved during maturation process by malolactic fermentation and maturation on oak chips. Malolactic fermentation can be considered as part of maturation process (Buglass, 2011). Oenoococcus oeni are the main bacteria responsible for malolactic fermentation (Fugelsang, 1997) converting L-malic acid to L-lactic acid and CO₂. Bacteria’s can adapt to rough environment of wines with high alcohol content, low pH and presence of sulphur dioxide (Lonvadj-Funel, 1999). In cider production malolactic fermentation is significant, and usually it starts after alcoholic fermentation. Exception is Asturian ciders because there both fermentation processes occurs simultaneously (Blanco Gomis, 2003) and efficiency depends on temperature and nutrients. In ciders from unpasteurized and unshulsured juices, can develop wild lactic acid bacteria (Lea, Drilleau, 2003). Winemakers assess the benefits of controlled malolactic fermentation (Krieger-Weber, 2009). Inoculation of starter, that mainly contains O. oeni bacteria, winemakers can reduce risks related to potential bacterial or bacteriopfage spoiling, and accelerate beginning of malolactic fermentation and harmonize wine taste and fullness.

In traditional winemaking fermentation and/or maturation occurs in oak barrels and it influence wine quality positively. Wine is fortified with substances extracted from oak, forming more complex aroma and taste of wines (Navojška et al., 2012). Oak barrels are expensive, takes up much area, and also it is more difficult to clean them. Based on these statements alternative methods for improvement of wine quality (Rodríguez-Rodríguez et al., 2011; Navojška et al., 2012) are developed, for example, use of wood chips. In England small and medium cider producers still use oak barrels and use of chips are not so popular comparing to wine industry (Buglass, 2011). Oak chips are obtained from wood processing byproducts, by using tradicional methods of treatment – boiling and roasting (Bozalongo et al., 2007). Fan et al. (2011) investigations showed influence of different oak chips (different raw materials, rolling degree) to the volatiles in cider. Oak typical aroma is influenced by wine and wood contact duration, temperatures, wood properties, for example, species, geographic origin, roasting degree (Garde-Cerdán, Ancín-Azpilicueta, 2006; Garde-Cerdán, 2010; Navojška et al., 2012). Several compounds are typical for unroasted oak (cis oak lactones) and others as vanillin, 4-methylguaiacol and furfural, mainly are formed from oak polymers that degrades and hydrolyses during storage (Hale et al., 1999). High roasting temperature influence degradation of wood polymers as lignins and cellulose, forming aldehydes, phenols, furfural derivatives, lactones (Nonier et al., 2006). In experiments about oak influence to the aroma of wine, positive correlation between concentration of vanillin and smoke and cinnamon aroma were determined (Spillman et al., 1998). Content of total phenolic compounds increases by increasing roasting intensity (Cabrita et al., 2011). Development and improvements of technology should be designed taking into account sensory evaluation. In order to match the instrumental analysis with the consumer requirements, a sensorial profile of the cider
is necessary. Sensory quality is related to consumer acceptance and confidence in the product, being defined by the interaction between the food and the consumer. Thus, sensory quality depends on both the sensory characteristics of the food and how consumers perceive them (Cardello, 1995; Costell, 2002; Ares et al., 2009). Sensory descriptive analysis is a primary tool of food scientists, which involves the evaluation of both the qualitative and quantitative sensory characteristics of products (Meilgaard et al., 1999).

The aim of current research was to evaluate influence of lactic acid bacteria _Oenococcus oeni_ and oak chips on the quality of apple cider.

**Materials and Methods**
Experiments were carried out at the Faculty of Food Technology, Latvia University of Agriculture in 2012.

**Materials**

Apples grown at the Latvian State Institute of Fruit Growing were used in the experiments. Apples were harvested and stored for 1–2 weeks at +3±1 °C with relative ambient humidity of 90–95%. In the present study juices of three varieties ‘Auksis’, ‘Lietuvas Pepins’., ‘Kerr’ in proportion 2 : 1 : 2 (by volume) were used. Fermentation was performed using the commercial _Saccharomyces cerevisiae_ yeast strain ‘71B-1122’ (Lalvin, Lallemard Inc., Canada). Fermentation was carried out at 16±1 °C for 28 days. In order to reduce the sharp acidity of cider (malic acid) lactic acid bacteria (Bacillus Malolactic Bacteria Culture _Oenococcus oeni_) (Lalvin, France) was added to the cider at a concentration of 0.05 g L⁻¹ (7.3×10⁵ cells L⁻¹). In order to improve the taste and aroma of cider, two types of oak chips were added:
- unroasted oak chips ‘French Oak Chips’ (Young’s Brew, UK);
- medium roasted oak chips ‘American oak chips’ (Browland, Belgium).

Oak chips were added in concentration 1.5 g L⁻¹ of cider. All cider samples were matured at +16±1 °C for four weeks.

In experiment the following samples were analysed:
- control – control sample stored without additives;
- LAB – sample matured with addition of _Oenococcus oeni_;
- U_OC – sample matured with unroasted oak chips;
- MR_OC – sample matured with medium roasted chips.

**Determination of total acids, solids and alcohol content**

Determination of titratable acidity (expressed as total acids) was performed according to method LVS EN 12147:2001 and expressed in g L⁻¹. Alcohol content was determined by volume % (FOCT 12787–81) and solids were determined after removal of alcohol gravimetrically.

**Determination of total phenolic content**
The total phenolic concentration was determined spectrophotometrically according to the Folin-Ciocalteu colorimetric method (Singleton, 1999). Cider was diluted with ethanol/acetic acid solution (1:20 v/v). The ethanol/acetic acid solution was prepared using an acetic acid water solution (2.5%) and ethanol (98% vol.) in ratio the of 10:90 (v/v). To 0.5 mL of aliquot 2.5 mL of Folin-Ciocalteu reagent (diluted 10 times with water) was added and, after 3 minutes 2 mL of sodium carbonate (Na₂CO₃) (75 g L⁻¹) was added. After 30 minutes of incubation at room temperature, the absorbance was measured at 765 nm using a JENWAY 6300 spectrophotometer (Baroworld Scientific Ltd., UK). Results were expressed as chlorogenic acid equivalents.

**Determination of individual polyphenols**
The concentration of all individual polyphenols was determined by High-performance liquid chromatography (HPLC) Shimadzu LC-20AD Prominance with diode array detector (SPD-M20A). Separation was performed in a PerkinElmer C18 4.6 mm×250 mm column (thermostated at 27 °C). Eluting solvents are methanol (A, 20%), water (B, 78.4%) and acetic acid (1.6%) used in a gradient mode and at 17.50 minutes solvents ratio are as follows – A concentration 40.3%, B concentration 58.5%, C concentration of 1.2%. 10 μL of the sample was injected in the chromatograph using automatic sample injection system SIL-20 AC. The total duration of the analysis was 35 minutes. For the detection and quantification of compounds, several wavelengths were used: 253 nm for 4-hydroxybenzoic acid and rutine, 263 nm for gallic acid, 278 nm for catechin, caffeic acid, syringic acid, 298 nm for chlorogenic acid, epicatechin, coumaric acid, sinapic acid and ferulic acid.

**Determination of volatile compounds**

Volatiles from ciders were extracted using solid phase microextraction (SPME). 5 g of cider were weighed in a 20 mL headspace vial and capped with a septum. A divinylbenzene/carboxen / polydimethylsiloxane (DVB/Car/PDMS) fiber (Supelco Inc., Bellefonte, PA, USA) was used for headspace SPME sampling. SPME parameters were: incubation time 30 min, extraction temperature 22±2 °C, extraction duration 30 min, desorption 15 min, 250 °C. For the analysis of the SPME extracts, a Perkin Elmer Clarus 500 GC/MS and a Elite-Wax ETR (60 m×0.25 mm i.d.; DF 0.25 μm) was used. Working conditions were: injector 250 °C; transfer line to MSD 260 °C; oven temperature start 50 °C, hold 2 min, programmed from 50 to 100 °C at 5 °C min⁻¹ hold 5 min, and from 100 to 210 °C at 5 °C min⁻¹, hold 15 min; carrier gas (He) 1 mL min⁻¹; split ratio 2:1; ionization EI⁺; acquisition parameters in full scan mode: scanned m/z 50–300. Compounds were identified by comparison of their mass spectra with mass spectral libraries (Nist98), and by calculation of linear retention indexes and comparison with literature data. All analyses were performed in triplicate. As a
Sensory analysis

Sensory evaluation of the ciders was carried out with trained panellists (33 women and 3 men, aged 21–71). The panellists had studied the basics of sensory evaluation methods and were experienced in sensory panels. This group included students and staff of the Latvia University of Agriculture Faculty of Food Technology. Line scale (ISO 4121:2003) for determination of the intensity of sensory properties (clarity, apple, fruit and yeast aroma, apple, yeast, sour, astringent and bitter taste) was used. Ranking test (ISO 8587:2006) was used to rank samples according to their degree of liking.

Statistical analysis

The analysis of variance was performed by the ANOVA procedure and p<0.05 was considered as statistically significant. Principal component analysis was performed with the software Multibase 2014 for Windows.

Results and Discussion

Sensory properties of cider can be improved by the maturation process using different technologies. Quality parameters of matured ciders are presented in Table1.

Table 1. Quality parameters of matured ciders

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total acids, g L⁻¹</th>
<th>Solids, g L⁻¹</th>
<th>Alcohol, vol %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.85±0.31ab</td>
<td>0.87±0.06b</td>
<td>5.58±0.23ab</td>
</tr>
<tr>
<td>LAB</td>
<td>3.99±0.21a</td>
<td>0.65±0.08a</td>
<td>5.60±0.21b</td>
</tr>
<tr>
<td>U_OC</td>
<td>7.16±0.25b</td>
<td>0.85±0.05b</td>
<td>5.52±0.25a</td>
</tr>
<tr>
<td>MR_OC</td>
<td>6.97±0.19b</td>
<td>0.84±0.06b</td>
<td>5.54±0.20ab</td>
</tr>
</tbody>
</table>

* The different letters in the same column represents significant differences between values (p<0.05).

In cider LAB significantly (p<0.05) lower total acid content and higher pH was detected. Also dry matter in this sample is significantly lower than in all other samples. The alcohol content and pH are important factors affecting the growth of lactic acid bacteria and their activity. Solier et al. (2010) study shows that the low pH of the wine significantly (p<0.05) negative effects malolactic fermentation. Gockowiak and Henschke (2003) study have shown that the pH from 2.9 to 3.5 have a negative impact on the viability of bacteria, but at pH 3.5 malolactic fermentation takes place successfully, regardless of the alcohol content. But the range of pH from 3.0 to 3.2 together with an alcohol content of 78.9 and 102.6 g L⁻¹ inhibited the lactic acid bacteria (Solier et al., 2010). Addition of oak chips to maturing process significantly (p<0.05) influenced only the content of total acids.

The total phenolic content in the analyzed ciders varied from 1028 mg L⁻¹ in the sample LAB to 1526 mg L⁻¹ in the sample MR_OC; the total phenolic content in the samples matured with oak chip was significantly (p<0.05) higher (Fig.1.).

Chlorogenic acid was the most important identified phenolic compound in all samples of ciders (Tab. 2). The highest content of chlorogenic acid was identified in the control sample, but the lowest content was identified in the sample LAB. Cabrita et al. (2008) in the research on wines revealed that the content of gallic acid, ferulic acid and caffeic acid increased after malolactic fermentation, but vanillin and syringic acid remained stable. The research of Figueiredo-González et al. (2014) showed that the content of catechin and epicatechin decreased during wine maturing in oak barrels. The sample MR_OC had significantly higher content of caffeic acid, epicatechin, ferulic acid and vanillin. At high temperature during roasting process lignina nd cellulose polymers contained in oak chips degrades forming aldehydes, phenols, furfural derivatives, lactones and other compounds (Nonier et al., 2006). Similarly, Bozalongo et al. (2007) found that oak roasting increased the content of compounds created by lignin thermal degradation (vanillin, eugenol etc.).

The highest content of 4-hydroxybenzoic acid was identified in the sample U_OC that corresponded to the findings of Cadahía et al. (2009) where the content of 4-hydroxybenzoic acid and its derivatives increased when wines were matured with French oak tree chips. In analysed samples 22 volatiles were identified. The highest total peak area for sample U_OC and the lowest peak are for control sample were detected. Main volatile compounds are alcohols forming 55.5% (LAB) to 69.1% (U_OC) and esters forming 25.5% (control) to 39.1% (LAB) from total peak area of identified compounds.

The analysis of variance showed a significant difference (p<0.05) in all sensory properties intensity except for the intensity of fruit aroma. The addition of lactic acid bacteria caused less distinct clarity in matured ciders with the most intensive apple aroma and taste, but with the least astringent, yeast and sour taste intensity.

The cider samples matured with unroasted and medium-roasted oak chips showed higher bitter taste intensity and lower sour taste intensity (Figure 2).
### Table 2

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Control</th>
<th>LAB</th>
<th>U_OC</th>
<th>MR_OC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorogenic acid</td>
<td>191.25±9.56bd</td>
<td>167.08±7.96a</td>
<td>190.69±9.08bd</td>
<td>188.41±6.28b</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>6.10±0.29 b</td>
<td>4.55±0.23 a</td>
<td>6.96±0.33 b</td>
<td>23.83±0.88c</td>
</tr>
<tr>
<td>Syringic acid</td>
<td>7.03±0.25 b</td>
<td>6.38±0.24 a</td>
<td>8.20±0.30c</td>
<td>6.20±0.31 a</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>0.10±0.01</td>
<td>n.i.</td>
<td>0.60±0.02</td>
<td>0.38±0.01</td>
</tr>
<tr>
<td>Vanillin</td>
<td>0.44±0.02 a</td>
<td>0.82±0.03 b</td>
<td>0.86±0.04 b</td>
<td>1.61±0.07 c</td>
</tr>
<tr>
<td>Hydroxybenzoic acid</td>
<td>0.30±0.02 b</td>
<td>0.17±0.01 a</td>
<td>0.86±0.03d</td>
<td>0.35±0.01 c</td>
</tr>
<tr>
<td>Catechin</td>
<td>2.82±0.12 d</td>
<td>3.26±0.13 c</td>
<td>0.78±0.03 a</td>
<td>1.18±0.06 b</td>
</tr>
<tr>
<td>Epicatechine</td>
<td>0.27±0.01d</td>
<td>0.08±0.01 b</td>
<td>0.05±0.01 a</td>
<td>3.16±0.14 c</td>
</tr>
<tr>
<td>Sinapic acid</td>
<td>0.11±0.01</td>
<td>0.31±0.01</td>
<td>n.i.</td>
<td>2.21±0.08</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>0.63±0.02 a</td>
<td>1.33±0.06 d</td>
<td>0.72±0.04 b</td>
<td>3.67±0.17 c</td>
</tr>
</tbody>
</table>

n.i. – not identified  
* The different letters in the same row represent significant differences between values (p<0.05).

Principal component analysis of volatile compounds and sensory properties showed that differences can be explained by three factors: the first two of them accounted for 82% of the total variable set (Fig. 3). The results showed that different cider maturing technologies influenced the content of volatile compounds in ciders. The control sample was characterized by the most intensive yeast aroma. The LAB sample was characterized by the most intensive fruit, apple aroma and the most intensive apple taste. The dominating volatile substances in the LAB sample were acetic acid, ethyl-9-decanoate, ethyl decanoate, octanoic acid, ethyl hexanoate; on the whole sweet, oily, fruit (grape), flower aromas, dominated in this sample; less dominant aromas were stale, bitter, soap and wax.
The ranking test results are presented as histograms showing frequency of each ranking score (1 – the highest rank; 4 – the lowest rank) evaluated by panellists (Figure 4). Preference ranking test results showed that the assessors preferred cider LAB.

Conclusions
Addition of lactic acid bacteria Oenococcus oeni during cider maturing process changed ciders’ chemical properties reducing the total content of acid, soluble solids, phenols, content of volatile compounds. Ciders matured with unroasted chips and medium-roasted chips have lower total content of acids and higher total phenolic content. Maturing with oak chips as well as addition of lactic acid bacteria influenced the ratio of volatile substances, i.e., content of esters and volatile acids increased, but content of alcohol decreased. Ciders matured by adding lactic acid bacteria Oenococcus oeni have the most intensive apple aroma and taste, the lowest astringent, yeast and sour taste intensity. The cider sample matured by unroasted and medium-roasted oak chips showed higher intensity of bitter taste and lower intensity of sour taste. The ranking test sensory results showed that the highest rank was awarded to the sample that had matured by adding lactic acid bacteria Oenococcus oeni.

Acknowledgment
State Research Programme NatRes Project no. 3. „Sustainable use of local agricultural resources for development of high nutritive value food products (Food)” and ESF project “Support for the implementation of LLU doctoral studies” contract No. 2009/0180/1DP/1.1.2.1.2/09/IAA/VIAA/017. The authors also acknowledge the Latvia State Institute of Fruit Growing for supplying us with apples.

References
BERRY AND FRUIT JUICES AS POTENTIAL UNTRADITIONAL ACIDITY REGULATORS IN MASHING

Ingmars Cinkmanis, Sanita Vucane, Ilze Cakste
Department of Chemistry, Faculty of Food Technology, Latvia University of Agriculture, Liela street 2, Jelgava, Latvia, e-mail: Ingmars.Cinkmanis@lla.lv

Abstract

Acids traditionally used for acidification of mash (lactic acid, phosphorus acid) provide optimal medium pH, however, it is theoretically possible to choose such agents that would complete several tasks, ensuring the regulation of pH. Berry and fruit juices (cranberry, black currant, red currant, quince, apple and lemon) containing different organic acids, such as citric acid, malic acid, tartaric acid and fumaric acid, have similar properties, although they can not only acidify mash but also increase the content of extract substances in wort. In berries and fruits juices titratable acidity and pH was measured potentiometrically using pH meter. The highest titratable acidity of berry and fruit juices was in lemon (5.71 mmol L⁻¹) and quince juice (5.80 mmol L⁻¹). Lemon juice has a lower pH 2.40 and apple juice has the highest pH 4.82. Results of the analysis of mash pH changes showed, that it is possible to reduce pH replacing traditional acidification regulators (lactic acid, phosphoric acid) with berry and fruit juices. The pH was practically in all the mashing stages in the limits of 5.14±0.02 up to 5.19±0.02. The content of wort extract was analyzed using beer analysing system – Anton Paar „Alcolaizer“ analysis. Using HPLC the Carbohydrates like glucose and maltose in wort were detected and quantified. The optimal berry and fruit mashing acidification regulators were quince and cranberry juices.

Keywords: acidity regulators, pH, mashing, mash, wort.

Introduction

In brewing, the raw materials that give the beer its specific sensory properties are water, barley malt, hop, and yeast. The most important technological process in producing of wort is the mashing what is the first stage of beer preparing (Heyse, 1995., Kunze, 1998). The factors that affect the output of malt extract are the quality of water, medium pH and temperature. In the process of mashing, it is important to provide the optimal conditions for starch hydrolysis ferments α-amylase and β-amylase activity. The largest α-amylase activity was stated when medium pH was 5.1–5.2. In the mashing process mash pH is higher and optimal pH is obtained by adding traditional acidificators: phosphor acid, lactic acid or gypsum to the mixture (Kunze, 1998; Narziß, 2004; Меледина et al., 2006). However, these traditional materials can be partially replaced with other products. Berry and fruit juices (cranberry, black currant, red currant, quince, apple and lemon) containing different organic acids, such as citric acid, malic acid, tartaric acid and fumaric acid, have similar properties, although they can not only acidify mash but also increase the content of extract substances in wort. The aim of the research was to produce the wort using untraditional acidity regulators as fruit and berry juices and to analyse its chemical composition.

Materials and Methods

Acidity regulators

Samples of berry and fruit: (black currant (Ribes nigrum), red currant (Ribes rubrum), quince (Cydonia oblonga), cranberry (Vaccinium microcarpum), apple (Antonovka) (Malus sylvestris) were collected at Cesis region in Latvia. Lemon juice is obtained from Lemon samples (Citrus x lemon) buy in local retail market Maxima. After collection, the berries and fruits were extracted in juice extractor Moulinex A7534K, and pasteurized 80 °C, 1.5 L min⁻¹ with Voran PA 90. Chemical acidity regulator reagents: lactic, phosphoric, fumaric, quinic, malic and citric acids HPLC–grade>99.0% (Fluka, Germany)

An amount of acidity regulators (Fig. 1.) added to experimental mash with initial pH.

Figure 1. An amount of added acidity regulators in mash

P – lactic acid, F – phosphoric acid, U – black currants juice, J – red currants juice, Cit – lemon juice, Cid – quince juice, Dz – cranberries juice, A – apple juice

Raw materials of mashing

Light barley malt (Brupak, England), water (SIA „Griģis un Co”) in accordance with the law of LR MK Nr.235/29.04.2003.

The titratable acidity and pH

The titratable acidity and pH was measured potentiometrically using pH meter – WTW inoLab® pH 720.

Wort extract

Content of wort extract was analysed using beer analysing system - Anton Paar „Alcolaizer analysis“.
Carbohydrates

The Carbohydrates was determined by HPLC using Shimadzu LC20 Prominence chromatographer. Determination parameters: Refractive Index Detection RID-10A; Column: Alltech NH₂, 4.6x250mm, 5μm; Temperature of the column: +30 °C; Mobile phase: A – acetonitrile and water (70:30); Isoctratic regime; Capacity of the injection sample: 10 μL; Rate of the flow: 1.3 mL min⁻¹; Total time of the analysis: up to 20 minutes.

Technological process of mashing and obtaining wort (Fig.2.)

1) Water (t=37 °C) (1 L) mixed with light malt (0.2 kg)
   pH of Mash 6.20 and acidificated to pH 5.20 with:
   1) Lactic or phosphoric acid
   2) Fumaric, quinic, malic or citric acid

2) Mashing:
   a) Mashing-in (τ=20 min, t=35 °C)
   b) Protein pause (τ=25 min, t=55 °C)
   c) Maltose pause (τ=40 min, t= 65 °C)
   d) Dextrin pause (τ=40 min, t=72 °C)

Mathematical data processing

Data obtained were analysed using statistical software Minitab 15 for Windows.

Results and Discussion

The titratable acidity and pH of berry and fruit juices.

The pH in the samples is characterized in Figure 3.

It should be concluded from the data presented in Figure 3, that from the berry and fruit juices the lemon juice has a lower pH 2.40 and apple juice has the highest pH 4.82.

The total titratable acidity of berry and fruit juices are showed in Figure 4.

Changes of pH in mashing process and evaluation of extract substance content

It is possible to reduce pH mash during mashing using cranberry, black currant, red currant, quince, apple, lemon fruit and berry juices up to pH 5.20±0.02. The changes of mash pH by fruit and berry juices are slight and vary between 5.19±0.02 and 5.14±0.02, thus providing amylase activity of α- and β-enzymes and optimal conditions for starch hydrolysis (Kunze, 1998, Montanari et al., 2005).

The content of measured extract substances in wort obtained during the experiments using traditional and untraditional acidity regulators is displayed in Fig. 5.

Optimal results of extract substances are gained using cranberry (12.5 Pl°) and quince (12.5 Pl°) juices, the lowest results – using red currant 9.9 (Pl°) and black
currant juices. The obtained results using traditional acidity regulators, lactic acid and phosphorus acid, are 12.0 Pl° and 10.5 Pl° (Fig. 5.). Comparing quinic and cranberries juices to the traditional acidity regulators lactic acid, the quinic and cranberries juice’s acquisition of extract substances is more efficient by 0.5 Pl°. Using lemon, apple, black currant and red currant juices for acidification of mash there were fewer amounts (by 14.4–23.2%) of extract substances extracted comparing to cranberry and quince juices.

As the amount of extract substances is different when using various acidity regulators in mashing process (Fig. 5), the concentration of maltose and glucose also differ significantly (Table 1 and 2). There was also a little amount of fructose (0.07–0.13%) and sucrose (0.26–0.54%) found in wort (Table 1 and 2). There was no fructose found in wort extracted using lactic and phosphorus acids, because the fructose come from juices. Acidity regulators – black currant, red currant, lemon, quince and cranberry juices – themselves contain fructose and glucose (Table 3).

The obtained data (Table 3) show that the content of fructose in black current (4.29%) and apple (3.60%) and juice in black current (4.29%) and apple (3.60%) and cranberry (1.64%) juices is the largest comparing to lemon (1.62%), quince (1.51%) and cranberry (1.64%) juices. The amount of glucose is also bigger in black current and red currant juices (3.80–3.66%), however the glucose amount in apple juice is the least (0.50%). Black currant and red currant juices contain 52–58% more glucose and 35–38% more fructose that lemon, quince and cranberry juices. The total content of glucose and fructose is arranged as follows: black current (8.09%) > red current (7.64%) > apple (4.10%) > cranberry (3.93%) > lemons (3.82%) > quince (3.51%).

---

**Table 1**

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Acidity regulators</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
</tr>
<tr>
<td>Fructose</td>
<td>–</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.36±0.03</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.40±0.06</td>
</tr>
<tr>
<td>Maltose</td>
<td>7.38±0.05</td>
</tr>
</tbody>
</table>

P – lactic acid, F – phosphoric acid, U – black currants juice, J – currants juice

**Table 2**

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Acidity regulators</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cit</td>
</tr>
<tr>
<td>Fructose</td>
<td>0.08±0.05</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.19±0.03</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.34±0.06</td>
</tr>
<tr>
<td>Maltose</td>
<td>6.50±0.05</td>
</tr>
</tbody>
</table>

Cit – lemon juice, Cid – quinic juice, Dz – cranberries juice, A – apple juice

---

**Table 3**

<table>
<thead>
<tr>
<th>Berry and fruit juices</th>
<th>Fructose</th>
<th>Glucose</th>
<th>Total content of glucose and fructose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black currants</td>
<td>4.29±0.21</td>
<td>3.80±0.19</td>
<td>8.09±0.20</td>
</tr>
<tr>
<td>Red currants</td>
<td>3.95±0.19</td>
<td>3.69±0.18</td>
<td>7.64±0.18</td>
</tr>
<tr>
<td>Apples</td>
<td>3.60±0.18</td>
<td>0.50±0.02</td>
<td>4.10±0.10</td>
</tr>
<tr>
<td>Cranberries</td>
<td>1.64±0.08</td>
<td>2.29±0.11</td>
<td>3.93±0.09</td>
</tr>
<tr>
<td>Lemons</td>
<td>1.62±0.08</td>
<td>2.20±0.11</td>
<td>3.84±0.09</td>
</tr>
<tr>
<td>Quince</td>
<td>1.51±0.07</td>
<td>2.00±0.10</td>
<td>3.51±0.08</td>
</tr>
</tbody>
</table>
Summarizing the results, the biggest amount of glucose and fructose is found in black currant and red currant juices, but the least – in quince juice.  

**Fumaric, quinic, malic and citric acids like acidity regulators**  
Due to the fact that berry and fruit juices differ because of various qualitative and quantitative content of acids, the following hypothesis has appeared: high outcomes of extract substances are connected with the concentration of malic acid and quinic acid in berry and fruit juices. Dominant acid in black currant, red currant and lemon juices is citric acid. Cranberry and quince juices contain not only citric acid, but also malic acid, quinic acid, but quince also contains fumaric acid. In order to verify the hypothesis that dominant organic acids that significantly affect the outcome of extract substances are malic acid and quinic acid, separate addition of these acids, citric acid and fumaric acid in mashing process was performed (Fig. 6). The obtained data displayed that added organic acids affect the content of glucose and maltose in wort to a great extent. The highest amount of glucose and maltose was extracted using quinic acid and malic acid as acidification agents, where the total amount of carbohydrates was 9.9±0.04% and 9.5±0.04%.

Conclusions  
The lemon juice has a lower pH 2.40 and apple juice has the highest pH 4.82. The highest titratable acidity of berry and fruit juices was in lemon (5.71 mmol L⁻¹) and quince juice (5.80 mmol L⁻¹). The changes of mash pH by fruit and berry juices are slight and vary between 5.19±0.02 and 5.14±0.02. Optimal results of extract substances are gained using cranberry (12.5 Pl°) and quince (12.5 Pl°) juices, the lowest results – using red currant 9.9 (Pl°) and black currant juices. The highest amount of glucose and maltose was extracted using tartaric acid and malic acid as acidity regulators, where the total amount of carbohydrates was 9.5±0.04%. Slightly smaller content of carbohydrates was measured when acidifying mash with fumaric acid (9.2±0.04%). Comparing quinic acid, malic acid and citric acid as acidity regulators together with regulation of pH these acids also affect the content of glucose and maltose, using citric acid 26.7% smaller amount of total carbohydrates content is extracted. Thus, experiments helped to find out that using lemon, apple, black currant and red currant juices for mash acidification it is possible to extract less amount of extract substances than by using cranberry and quince juices, as the dominant acid in lemon, black currant and red currant juices is citric acid.

**References**  
QUALITY CHANGES OF NATURALLY FERMENTED KVASS DURING PRODUCTION STAGES

Ivo Lidums, Daina Karklina, Asnate Kirse

Department of Food Technology, Faculty of Food Technology, Latvia University of Agriculture, Liela Street 2, Jelgava, Latvia, e-mail: ivo@riela.lv

Abstract

Commercially available beverages sold as kvass are kvass drinks and malt extract drinks, made by diluting grain extract concentrates with water and adding colourings, different flavours and artificial sweeteners. Kvass quality parameters are defined by the Regulation No 926/2010 Quality and classification requirements for kvass and kvass (malt) beverage of the Cabinet of Ministers of the Republic of Latvia Naturally fermented kvass is made from rye bread rusks without additional additives. The aim of this research was to assess the quality changes of naturally fermented kvass during production stages. Experiments were carried out at the Latvia University of Agriculture Department of Food Technology. Dry matter (refractometer, ISO 6496), active acidity (LVSE 10523:2012) and sensory properties (25 panellists; line scale ISO 4121:2003) were assessed in kvass samples during production stages. During fermentation stage of naturally fermented kvass, pH drops from 4.08 to 3.77 and in later production stages pH is between 3.82 and 3.88, pH levels do not exceed the index values of the Regulation of the Cabinet of Ministers. Relative dry matter content reduced from 5.96% to 4.94%. Sensory evaluation showed that the intensity of flavour, aroma and acidity was most pronounced in kvass sample C (total production time 156 h), however, colour was most pronounced in kvass sample A (total production time 36 h). Longer maturation process aids in the formation of more robust flavour as well as yeast and protein residue.

Keywords: kvass, dry matter, active acidity, sensory evaluation.

Introduction

Kvass is a non-alcoholic beverage that can be used without restriction, its effects on the human body is similar to kefir. This is due to lactic acid which is formed by lactic acid bacteria (Costa et al., 2013). Kvass has beneficial effects on the digestive tract (Feik et al., 2010), furthermore energy value of naturally fermented kvass is only 25 kcal (105 kJ) per 100 mL (Costa et al., 2013). Kvass has thirst soothing and diuretic properties. Fermented bread kvass has the positive qualities of beer, as well as minerals and vitamins. It contains more than 30 minerals and trace elements. Kvass contains such minerals as copper, phosphorus, potassium, zinc, iron, and fluorine and B vitamins – thiamine, riboflavin, niacin and folic acid (Zariņš, 1991); the content of thiamine (B₁) in naturally fermented kvass is up to 0.04 mg per 100 mL (Покровский, 1976). There are no fat, cholesterol and nitrates in kvass. Most of the beneficial substances come from the raw materials used in naturally fermented kvass production – rye bread and malt. Kvass is very low in sodium, so it promotes the excretion of fluid and it can recommend instead of other soft drinks for people who want to lower their blood pressure with food restrictions (Рудольф, 1982). Commercially available beverages sold as kvass are kvass drinks and malt extract drinks, made by diluting grain extract concentrates with water and adding colourings, preservatives, different flavours and artificial sweeteners. The most commonly used preservatives in soft drink production are benzoic acid, sorbic acid and sodium benzoate (Джорданов, 1990). Regulation No 926/2010 of Cabinet of Ministers of the Republic of Latvia indicates that it is allowed to use these raw materials if they comply with the requirements set in food laws and regulations for kvass production: spring water, drinking water, fruit juice and puree, vegetable juice and puree, fruit juice concentrate, sugar, kvass mash concentrate, bread rusks, cereals, malt and grain products, raw plant extracts, kvass concentrate, carbon dioxide, compressed bakers’ yeast or cultured yeast, honey, sweeteners and flavourings (except in the production of kvass), food additives.

Maximum cleanliness and hygiene conditions associated with good manufacturing practice must be provided during kvass production therefore stainless steel tanks are used (Bidžane, 2000). Water quality affects the formation of kvass sensory indicators. Kvass consistency is better if softer water is used. Elevated sulphate content in the water causes kvass to taste bitter, silicates interfere with the fermentation process and causes sludge, chlorides lead to unpleasantly sweet taste, iron and manganese affect kvass colour and foaming (Hugenholzt, 2013). Malt for kvass production is usually obtained from spring barley, which has low levels of protein (8–11%) and higher levels of starch which contains the necessary sugars for fermentation. Germination of grains activates the break down starch and proteins during mash cooking (Sacher, 2013), thus obtaining necessary nutrients for yeasts and lactic acid bacteria. Rye bread rusks which are the basic raw material in naturally fermented kvass production have a strong flavour and sour taste. Rye bread rusks are obtained by drying sliced or diced rye bread. Bread yeast Saccharomyces cerevisiae is used for kvass production; yeast cells cause intense ethanol fermentation during anaerobic fermentation forming alcohol and carbon dioxide. To control fermentation and avoid formation of too much alcohol, oxygen is supplied during this process (Birch et al., 2013). Active lactic acid bacteria growth takes place simultaneously with yeast cell growth during mash fermentation; lactic acid bacteria produce lactic acid.
Bread kvass made from pure cultures of lactic acid bacteria and yeasts is clearer and has increased resilience. Yeast and lactic acid bacteria that give refreshing taste and aroma are most commonly used. (Dlusskaya et al., 2008) Malt extract and bread fermentation process provides proteins, sugars, organic acids and vitamins.

Usually, preservatives which extend shelf life are added or kvass pasteurization is used. These processes shorten production time and costs, and also impact such sensory properties as taste and smell, as well as biologically active compounds. Naturally fermented kvass is made without preservatives and is not pasteurized, thus saving the maximum quantity of vitamins and minerals, as well as the flavour and aroma.

Before the production of a new type of kvass it is necessary to define sensory scores, which can significantly affect consumer beverage choices. Therefore the aim of this research was to assess the quality changes of naturally fermented kvass during production stages.

Materials and Methods

All analyses were completed at Microbiology Research laboratory, laboratory of Sensory analyses and laboratory of Bread technology at Latvia University of Agriculture.

Kvass production

For the bread kvass production the following materials were used: rye bread rusks (Ltd Liepkalni), baker’s yeast *Saccharomyces cerevisiae* (Sp.z.o.o. Lallemand), lactic acid bacteria *Leuconostoc mesentericus* (Ltd Chr. Hansen), beet sugar (Ltd Dansukker) and dark malt (Ltd Liepkalni).

To prepare 1 litre of kvass mash, 200 g of rye bread rusks and 2 g dark malt were soaked in 2 litres of hot water (78±2 °C). Bread rusks were left to soak for 3 hours, then the water-bread rusk suspension was filtered (300 microns) and the liquid fraction was cooled down and used in further kvass production stages.

1 g baker’s yeast, 2 units of lactic acid starter and 30 % of the estimated quantity of sugar were added to 1 litre of kvass mash. The total quantity of sugar for kvass production is 30 g; therefore 10 g of sugar were added prior to fermentation. The fermentation of kvass mash took 9 hours at 27±1 °C.

After fermentation kvass was placed in a refrigeration chamber to cool down to 3±1 °C. After cooling, the yeasts were filtered (5 microns) and the remaining sugar was added (blending). Kvass was maturated for 12 hours at 6±1 °C and then it was ready for drinking (total production time 25 hours).

Physicochemical analyses

The Regulation No 926/2010 defines such kvass quality parameters:

1) dry matter content – 3.0 to 14.0 percent by weight,
2) acidity – 2.0 to 3.5, expressed as mL of 1N NaOH per 100 mL.

Active acidity (pH) and dry matter content was determined in kvass during 8 production stages (Table 1). Active acidity (pH) was determined according to the standard (AACC 02-31) and dry matter was determined with table refractometer according to the standard ISO 6496).

Microbiological analyses

Lactic acid bacteria were determined in kvass during 8 production stages (Table 1) according to the standard LVS ISO 15214:1998.

Sensory analyses

Kvass samples were evaluated sensory by 25 trained panellists (36% men and 64% women), average age 21 years.

Each panellist was served 3 samples of kvass in a randomized serving sequence: kvass stored for 11 h (sample A, total production time 36 h), kvass stored for 59 h (sample B, total production time 84 h), and kvass stored for 131 h (sample C, total production time 156 h). Line scale was used to evaluate the intensity of kvass sensory properties (aroma, flavour, acidity, and colour) (ISO 4121:2003). Kvass samples for sensory
evaluation were chosen according to organoleptic evaluation by research funder.

### Table 1

<table>
<thead>
<tr>
<th>Stage</th>
<th>Materials and technological process</th>
<th>Time, h</th>
</tr>
</thead>
<tbody>
<tr>
<td>S₀</td>
<td>Rye bread rusks, before soaking</td>
<td>0</td>
</tr>
<tr>
<td>S₁</td>
<td>Kvass after fermentation</td>
<td>12</td>
</tr>
<tr>
<td>S₂</td>
<td>Kvass after blending</td>
<td>13</td>
</tr>
<tr>
<td>S₃</td>
<td>Kvass after maturation</td>
<td>36</td>
</tr>
<tr>
<td>S₄</td>
<td>Kvass during storage</td>
<td>60</td>
</tr>
<tr>
<td>S₅</td>
<td>Kvass during storage</td>
<td>84</td>
</tr>
<tr>
<td>S₆</td>
<td>Kvass during storage</td>
<td>132</td>
</tr>
<tr>
<td>S₇</td>
<td>Kvass during storage</td>
<td>156</td>
</tr>
</tbody>
</table>

### Data analyses

The obtained data processing was performed with the Microsoft Excel 13 for Windows; arithmetic mean, standard deviation and standard error were calculated (Arhipova et al., 1999). For data cross-comparison ANOVA, Regression and other statistical calculation functions were used. Both t-test and F-test were used in order to assess the significance of changes and inter-comparison of the obtained data. For the interpretation of the results it is assumed that α=0.05 with 95% confidence (Næs et al., 2011).

### Results and Discussion

The changes detected in pH during kvass production stages are given in Figure 2.

The numerical value of pH decreased slightly during kvass fermentation; the initial pH was 4.08 and pH after fermentation was 3.77. During the rest stages of kvass production pH stabilized and was around pH 3.85. Compared to the indexed values of the Regulation No 926/2010, pH value of laboratory produced kvass corresponded to the regulatory scale (experimentally determined conversion factor approximately 1.8). pH value did not decrease to interval lower limit value (2.0 mL 1 N NaOH) in any of the controlled production stages; at the end a trend in pH increase was observed. The increase of pH in kvass at the end of production stages could be explained by the formation of new substances because yeast and lactic acid bacteria cells gradually die. A strong, negative correlation (r= -0.92) was observed between pH and lactic acid bacteria count changes in kvass. Correlation is significant (p<0.05) primarily during the first three stages of kvass production (Lidums, 2011).

During later stages, at the onset of lactic culture gradual degradation and stabilization of pH, correlation is weak and negative (r= -0.11). As pH is the logarithm of H⁺ ion concentration, correlation is compared to the logarithmic values of lactic acid bacteria count. A moderate, negative correlation (r = -0.50) was observed using pairwise correlation calculation between pH and the absolute number of lactic acid bacteria.

### Figure 2. Changes in pH and lactic acid bacteria count during kvass production stages

S₀ – rye bread rusks, before soaking, S₁ – kvass after fermentation, S₂ – kvass after blending, S₃ – kvass after maturation, S₄, S₅, S₆, S₇ – kvass during storage

During kvass production stages dry matter content (%) experienced a slight decrease at the expense of volatile fermentation product formation (mainly alcohol). At times a rise in dry matter content can be observed at the expense of increased fermentative microorganism total cell count. Decrease in dry matter content was found after intensive fermentation stage (S₁). Relative dry matter content changed from 5.96% to 4.94%. Decrease in the intensity of fermentation caused sedimentation of some substances that were not fully included in the test sample. In later stages of kvass production significant changes on dry matter content were not observed (p>0.05).

The changes in indicator value were characterized by this polynomial division:

\[
y = 0.00005x^2 - 0.0148x + 6.0285	ag{1}
\]

where y – the dry matter content (%) after a certain time, x – fermentation, h.

The process is characterized by regression R² = 0.99. The first right-hand number in the division (1) is a very low figure, so it can be dropped and the calculations performed with a linear response without significant mistakes:

\[
y = -0.015x + 50	ag{2}
\]

where y – the dry matter content, %; x – fermentation, h; 50 – initial dry matter content, %. It should be noted that the decrease in dry matter content affects physical as well as chemical and biogenic elements, so for each particular set of circumstances of kvass production stages a calculation formula must be found. In general form is looks like this:

\[
\text{Dry matter (\%)} = -k \times \text{(hours)} + \text{initial dry matter (\%)}	ag{3}
\]
were used for yeast and lactic acid bacteria development.
Since in kvass production normative documents no strict parameters have been set for acidity (affects flavour) and dry matter (affects clarity), solely the recommended value intervals, many versions and combinations (market brands) of kvass quality and sensory properties are possible.

The intensity of sensory properties of three kvass samples with different storage (total production) times was evaluated (Fig. 4).

Figure 3. Changes in dry matter content (%) during kvass production stages

Dry matter content decreased during kvass fermentation, as most of the dry ingredients and sugar were used for yeast and lactic acid bacteria development.

Because of further maturation happening during storage, yeasts left in kvass continue to ferment remaining sugars, resulting in increased acidity.

Conclusions

During production stages, changes in active acidity range from pH 3.77 to pH 4.08. Relative dry matter content reduced from 5.96% to 4.94%.

Sensory evaluation showed that the intensity of flavour, aroma and acidity was most pronounced in kvass sample C (total production time 156 h), however, colour was most pronounced in kvass sample A (total production time 36 h). Longer maturation process aids in the formation of more robust flavour as well as yeast and protein residue.

References
INFLUENCE OF FREEZING AND DRYING ON THE PHENOL CONTENT AND ANTIOXIDANT ACTIVITY OF HORSERADISH AND LOVAGE

Lolita Tomsone, Zanda Kruma

Department of Food Technology, Faculty of Food Technology, Latvia University of Agriculture, Liela Street 2, Jelgava, Latvia

e-mail: lolitatomsone@gmail.com

Abstract

A perennial herb lovage (Levisticum officinale L.) and horseradish (Armoracia rusticana L.) contains biologically active substances and are cultivated in temperate regions of the world. The aim of this work was to study the effect of the technological processes (freezing and drying) on the polyphenol content and antioxidant activity of horseradish leaves and lovage leaves and stems. The samples were processed using freezing (-20 °C) and drying (at temperature of +24 °C, in a dark) till moisture content 10±2%, and for a comparison fresh samples were analysed. Lovage leaves and stems and horseradish leaves were extracted with ethanol using conventional extraction. Total phenols, total flavonoids, antioxidant activity were determined spectrophotometrically, and individual polyphenolics were identified using HPLC. Analysis of the phenolic compounds and antioxidant activity of lovage and horseradish showed differences depending on the technological processes applied. The predominant phenolic acid in lovage samples was caffeic acid, but horseradish leaves – chlorogenic acid, and the major flavonoid was rutin. Only sinapic acid in lovage stems were found to be higher in the dried samples, compared to the fresh and frozen samples. From analysed methods better results for preserving phenolic compounds and antioxidant activity of lovage leaves and stems and horseradish leaves proved to be freezing.

Keywords: antioxidant, horseradish, lovage, phenolic, treatment

Introduction

World scientists have been paid attention to preservation of biologically active substances in food. Plants provide abundant natural antioxidants, which are vitally important for human health (Naczk, Shahidi, 2006).

Horseradish (Armoracia rusticana L.) and lovage (Levisticum officinale L.) are perennial plants which belong to Brassicaceae and Umbelliferae families, respectively. Horseradish is with a particularly pungent flavour, rich in vitamin C (302 mg 100 g−1) (Raghavan, 2000) All parts of lovage are strongly aromatic with a characteristic earthy, celery-like flavour and smell (Szebeni-Galambosi et al., 1992; Raghavan, 2000).

Both plants contain compounds that can act as natural antioxidants (Raghavan, 2000). The antioxidant characteristics of plant derived materials can be attributed to their polyphenols.

Phenolic composition of plants is affected by different factors – variety, genotype, climate, harvest time, storage, processing, and treatment (Marrelli et al., 2012). Changes of the content of biologically active compounds in plants can cause climate, soil, vegetative stage of the plant and also technological processes applied – drying, freezing and other heat treatment methods, irradiation etc. (Angela, Meireles, 2009).

Technological processes has important role in final product quality and especially to the antioxidant activity. Part of biologically active compounds are instable during technological processes applied, resulting in decrease of biological value of fruit and it is very important to find best methods for preserving their value.

Drying is widely used preservation method as the drying process inhibits enzymatic degradation and limits microbial growth (Ahrene et al., 2007) Drying method also influence the composition and biological activity of plant (Ahrene et al., 2007). Treatment at higher temperature decreases content of gallic acid (Orkoula et al., 2004), whereas during drying of sweet potatoes total phenolic content and antioxidant activity increases (Yang et al., 2010).

Results of investigations about influence of freezing and storage in frozen state to the changes of phenolic composition differ, and tendencies depend on food matrix used. Freezing has not significant influence to the content of elagic acid and total phenols content in raspberries (Ancos et al., 2000), but in frozen blueberries total phenols content decreases (Rizzolo et al., 2003).

Irish scientists reported that after processing of six Lamiaceae herbs (rosemary, oregano, marjoram, basil, thyme) content of rosmarinic acid increased (Hossain et al., 2010), similar trend a was found to polyphenolic compounds in horseradish roots (Tomsone et al., 2013).

The aim of this work was to study the effect of the technological processes (freezing and drying) on the polyphenol content and antioxidant activity of horseradish leaves and lovage leaves and stems.

Materials and Methods

Chemicals

Gallic acid, Folin-Ciocalteu phenol reagent, and 2,2-diphenyl-1-picrylhydrazili (DPPH*) were purchased from Sigma-Aldrich (Switzerland). All other chemicals (Na2CO3, ethanol) used in the research were obtained from Acros Organic (USA).

Sample preparation

Fresh horseradish leaves (Armoracia rusticana L.) and lovage leaves and stems (Levisticum officinale L.) were collected in Latvia in June 2013. The samples were processed using the following methods: a) freezing (-20 °C), b) drying (in a dark, temperature of +24 °C, till moisture content 10±2%).
Extraction procedure
The homogenized sample were extracted with ethanol in a conical flask with a magnetic stirrer (magnet 4.0×0.5 cm) at 700 rpm for 1 h at room temperature (20±1 °C). The extracts were then filtered (paper No. 89). The extraction process was done in triplicate.

Determination of total phenolic and total flavonoid compounds
The total phenolic content (TPC) of the plant extract was determined according to the Folin-Ciocalteu spectrophotometric method (Singleton et al., 1999) with some modifications. The absorbance was measured at 765 nm and total phenols were expressed as the gallic acid equivalents (GAE) 100 g⁻¹ dry weight (DW) of plant material. The total flavonoid content (TFC) was measured by a colorimetric method (Kim et al., 2003) with minor modification. The absorbance was measured at 415 nm and total flavonoids were expressed as the catechin equivalents (CE) 100 g⁻¹ DW of plant material.

Determination of antioxidant activity
Antioxidant activity of the plant extracts was measured on the basis of scavenging activities of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH⁺) radical as outlined by Yu et al. (2003). The absorbance was measured at 517 nm. The radical scavenging activity (RSA) of extract was also measured by 2,2′-azino-bis(3-ethylbenz-thiazoline-6-sulfonic) acid (ABTS⁺⁻) radical cation assay (Re et al., 1999). For the assessment of extracts, the ABTS⁺⁻ solution was diluted with a phosphate buffer solution to obtain the absorbance of 0.800±0.030 at 734 nm. The RSA was expressed as TE 100 g⁻¹ DW of plant material. The higher the Trolox equivalent antioxidant capacity (TEAC) of a sample, the stronger the antioxidant activity. The reducing power can be determined by the TEAC (TE) of a sample, the stronger the antioxidant activity. The reducing power was calculated according to the standard ISO 6986:1999 and all results were expressed on dry basis.

Reversed phase high performance liquid chromatography (HPLC) analysis of the extracts
The analyses were carried out using a Shimadzu liquid chromatograph LC-20AD with the analytical column C18, photo diode array detector SPD M20A. As eluting solvents were used methanol (A, 20%), water (B, 78.4%), and acetic acid (C, 1.6%) using a gradient mode: 17.50 minutes – 40.3% A concentration, 58.5% B concentration, C concentration of 1.2%, 35th minute till end. The sample injection into the chromatograph was performed using an automatic sample injection system SIL-20AC. Eluent flow rate was 1.0 mL min⁻¹. Several wavelengths were used to define polyphenols. Using wavelength 253 nm 4-hydroxybenzoic acid and rutine were determined; 263 nm – gallic acid; 278 nm – catechin, caffeic acid, syringic acid; 298 nm – chlorogenic acid, epicatechine, coumaric acid, sinapic acid, and ferulic acid.

Statistical analysis
Experimental results are means of three parallel measurements and were analyzed by Microsoft Excel 2010 and SPSS 17.00. Analysis of variance (ANOVA) and Tukey’s test were used to determine differences among samples. A linear correlation analysis was performed in order to determine relationship between TPC, TF, antioxidant activity such as DPPH⁺, ABTS⁺⁻ and reducing power. Differences were considered as significant at p<0.05.

Results and Discussion
Total phenolics and flavonoids content
The TPC and TFC determined in horseradish leaves and lovage leaves and stems extracts depending on treatment are shown in Table 1. Results of multivariate dispersion analyses showed that both type of treatment and plant material are significant factors affecting TPC and TFC (p<0.05). The highest content of phenolic compounds was determined in horseradish leaves.

<table>
<thead>
<tr>
<th>Plant material</th>
<th>Type of treatment</th>
<th>TPC, mg GAE 100 g⁻¹ DW</th>
<th>TFC, mg CE 100 g⁻¹ DW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horseradish leaves</td>
<td>Fresh</td>
<td>2368.48±2.03b</td>
<td>5889.85±6.02b</td>
</tr>
<tr>
<td></td>
<td>Frozen</td>
<td>2722.13±2.03a</td>
<td>6178.03±6.27a</td>
</tr>
<tr>
<td></td>
<td>Dried</td>
<td>123.60±0.17b</td>
<td>287.79±0.28b</td>
</tr>
<tr>
<td>Lovage leaves</td>
<td>Fresh</td>
<td>1593.61±1.09d</td>
<td>2965.40±2.69a</td>
</tr>
<tr>
<td></td>
<td>Frozen</td>
<td>1601.87±1.47c</td>
<td>3548.33±3.08c</td>
</tr>
<tr>
<td></td>
<td>Dried</td>
<td>359.75±0.37f</td>
<td>551.01±0.55f</td>
</tr>
<tr>
<td>Lovage stems</td>
<td>Fresh</td>
<td>381.15±0.39f</td>
<td>1208.18±1.83f</td>
</tr>
<tr>
<td></td>
<td>Frozen</td>
<td>458.03±0.40e</td>
<td>1259.94±1.51e</td>
</tr>
<tr>
<td></td>
<td>Dried</td>
<td>44.83±0.07e</td>
<td>184.04±0.14e</td>
</tr>
</tbody>
</table>

Processing technologies has similar influence to the content of bioactive compounds for all types of plant materials investigated. It is possible to observe that for all plant materials after freezing content of phenolic compounds increased and the highest increase in lovage stems was detected – 20%. Whereas in lovage leaves TFC in frozen samples are for 20% higher. The same tendency was also observed in investigations about fresh and frozen horseradish roots (Tomson et al., 2013). Similar results that at lower temperature increase TPC also were reported in Etlingera elatior and Morus alba L. (Chan et al., 2013). Obtained results could be explained by fact that ice crystals formed within the plant matrix can rupture cell structure. This allows the exit of cellular components and access of solvent (Asam et al., 2003). Whereas in maringold after freezing TPC and TFC slightly decreased.
Higher amounts of all phenolic compounds were found in fresh samples. The predominant phenolic acid in lovage samples was caffeic acid, but in horseradish leaves – chlorogenic acid. The highest content of chlorogenic acid was in the lovage stems. The major flavonoid was rutin, and highest content in fresh horseradish leaves were identified. Comparing results of TPC, TFC and individual phenolic compounds (Table 1 and Table 2) it is possible to conclude that horseradish and lovage leaves contain other flavonoids, and rutin forms only part of TFC. Also in horseradish roots rutin, caffeic acid and chlorogenic acid in significant amounts were identified (Tomson et al., 2013). Only sinapic acid in lovage stems were found to be higher in the dried samples, compared to the fresh and frozen samples. In research about horseradish roots higher content of rutin and caffeic acid in frozen samples were detected (Tomson et al., 2013). Also literature studies showed that each phenolic compound behave different depending on treatment method. Content of rosmarinic acid after freezing and drying decreases, but carnosic acid content in the same conditions increased (Mulinacci et al., 2011). Caffeic acid and ferulic acid both are hydroxycinnamic acids, and depending on treatment they behave different. In hara leaves content of caffeic acid after freezing increased, but ferulic acid has opposite effect (Lin et al., 2012). In literature it is found that phenolic...
compounds can fast degradates in high temperatures in oxygen presence (Igual et al., 2012). Spanish scientists reported that in red onions frozen with CO₂ and stored at temperature individual phenolic content decreases for 24% (Pérez-Gregorio et al., 2011). Also flavonoids content in rosmary leaves during freezing and drying decreased (Mulinnacca et al., 2011). Whereas in hara leaves better results were obtained for samples dried in room temperatures (Lin et al., 2012). Phenolic compounds may differ from one another with respect to their binding status, depending on specific aspects of their chemical structures. Thus, processes may differ in their effectiveness in liberating phenolic acids from plant tissues. Phenolic acids occur in plants as metabolic intermediates, and they also accumulate in the vacuoles (Chism, Haard, 1996). Thermal processing may release more bound phenolic acids due to the breakdown of cellular constituents. Although disruption of cell walls also releases the oxidative and hydrolytic enzymes that can destroy the antioxidants in fruits and vegetables (Chism, Haard, 1996), thermal processing deactivates these enzymes to avoid the loss of phenolic acids (Dewanto et al., 2002).

Antioxidant activity

Phenolic compounds are commonly found in plants and they have been reported to have strong antioxidant activities (Bors et al., 2001; Li et al., 2006). The antioxidant potential of phenolic compounds is dependent on the number and arrangement of the hydroxyl groups as well as the presence of the electron donating substitute in the ring structure (Elzaawely et al., 2007). It is considered that in plant material poor in vitamin C, main antioxidants are phenolic acids and flavonoids (Igual et al., 2012).

Results of multivariate dispersion analyses showed that both type of treatment and plant material are significant factors affecting (p<0.05) for antioxidant activity (Table 3). Secondary antioxidant activity (DPPH⁺ and ABTS⁻⁺) for all samples are similar tendencies.

<table>
<thead>
<tr>
<th>Plant material</th>
<th>Type of treatment</th>
<th>DPPH⁺, mM TE 100 g⁻¹ DW</th>
<th>ABTS⁺⁺, mM TE 100 g⁻¹ DW</th>
<th>Reducing power, mg AAE 100 g⁻¹ DW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horseradish leaves</td>
<td>Fresh</td>
<td>22.84±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>154.36±0.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9573.38±9.07&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Frozen</td>
<td>13.21±0.04&lt;sup&gt;d&lt;/sup&gt;</td>
<td>88.62±0.09&lt;sup&gt;d&lt;/sup&gt;</td>
<td>15994.88±13.02&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Dried</td>
<td>8.87±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.80±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2254.65±2.71&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lovage leaves</td>
<td>Fresh</td>
<td>10.90±0.02&lt;sup&gt;e&lt;/sup&gt;</td>
<td>98.66±0.07&lt;sup&gt;c&lt;/sup&gt;</td>
<td>21225.22±20.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Frozen</td>
<td>10.12±0.02&lt;sup&gt;f&lt;/sup&gt;</td>
<td>99.61±0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22857.82±22.06&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Dried</td>
<td>8.72±0.01&lt;sup&gt;f&lt;/sup&gt;</td>
<td>30.13±0.05&lt;sup&gt;f&lt;/sup&gt;</td>
<td>4196.27±4.08&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lovage stems</td>
<td>Fresh</td>
<td>21.25±0.06&lt;sup&gt;e&lt;/sup&gt;</td>
<td>32.50±0.05&lt;sup&gt;f&lt;/sup&gt;</td>
<td>4330.21±4.04&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Frozen</td>
<td>35.06±0.07&lt;sup&gt;e&lt;/sup&gt;</td>
<td>42.46±0.06&lt;sup&gt;e&lt;/sup&gt;</td>
<td>6684.49±5.94&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Dried</td>
<td>9.82±0.03&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3.98±0.01&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2389.65±2.43&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* Mean values within the same column followed by different letters significantly differ according to the LSD test (p<0.05).

Frozen lovage stem scavenging activity increases for 65%, and similar tendencies also were reported for horseradish roots (Tomsone et al., 2013). In horseradish leaves opposite tendency were observed, and scavenging activity decreases for 43% comparing to fresh samples. Increase of antioxidant activity could be explained by fact that splitting of molecular structure covalent complex deliberates antioxidant compounds as flavonoids, carotenoids, lycopene etc. (Siriamornpun et al., 2012). Many antioxidant phenolic compounds in plants are most frequently present in a covalently bound form with insoluble polymers (Siriamornpun et al., 2012).

In dried lovage leaves remains 80% of antioxidant activity, comparing to fresh material. In hara leaves DPPH radical scavenging activity after freezing is lower, comparing to drying at room temperatures (Lin et al., 2012). Decrease of antioxidant activity due to thermal degradation of phenolic compounds could be explained by to loss of antioxidant enzyme activities and activity of degradative enzymes (Lim, Murtijaya, 2007).

Similar like phenolic content, also primary antioxidant activity (reducing power) for all frozen samples are higher, compared to fresh samples. The same tendency for horseradish roots is observed (Tomsone et al., 2013). The highest increase in horseradish leaves and lovage stems were observed, 67% and 54%, respectively. Whereas after drying the higher primary antioxidant activity was maintained in lovage stem samples. Other authors reported similar tendencies in changes of antioxidant activity and TPC (Chan et al., 2009; Ahmad-Qasem et al., 2013).

Correlation between phenolic content and antioxidant activity

Phenolic compounds have radical scavenging activity. Correlation analysis was performed to determine relationship between these parameters. The antioxidative capacity of an extract is often related to its polyphenolic constituents. In our study all correlations between analysed samples are positive and the strongest correlation was for lovage stems (Table 4), but for horseradish and lovage leaves medium or weak correlations (data not shown).
For horseradish roots correlation between TPC and TFC was strong ($r=0.86$), but between different antioxidant assays are medium or weak (Tomson et al., 2013), that is similar to results of current research. Kubola and Siriamornpun (2008) studied bitter gourd (Momordica charantia L.) leaf, stem and fruit fraction and referred that correlation between TPC and antioxidant activity (DPPH) was moderate ($r=0.7$), and between TPC and primary antioxidant activity (Reducing Power) very strong ($r=0.95$), but antioxidant activity (DPPH) and primary antioxidant activity (Reducing Power) show moderate correlation ($r=0.55$). Statistical correlations between TPC and total antioxidant capacity of litchi seed extract were strong ($r=0.98$) (Prasad et al., 2009). Strong correlation between phenolic compounds and antiradical activity was also found in experiments about seabuckthorn (Hippophae rhamnoides L.) leaves (Kumar et al., 2011), lychee (L. chinensis Sonn.) flowers (Liu et al., 2009) and canola meal (Hassas-Roudsari et al., 2009).

Conclusions

Analysis of the phenolic compounds and antioxidant activity of the horseradish leaves and lovage stems showed differences depending on the technological processes applied. In order to select the best treatment method criteria such as phenolic compounds and antioxidant activity should be considered. The predominant phenolic acid in lovage samples was caffeic acid, but horseradish leaves – chlorogenic acid, and the major flavonoid was rutin. Only sinapic acid in lovage stems were found to be higher in the dried samples, compared to the fresh and freeze samples. Based on the total phenolic and flavonoid content, one of the best traditional methods for preserving phenolic compounds and antioxidant activity of the horseradish leaves and lovage leaves and stems proved to be freezing.

References


Table 4

<table>
<thead>
<tr>
<th>TPC</th>
<th>TFC</th>
<th>DPPH</th>
<th>ABTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>0.943**</td>
<td>1</td>
</tr>
<tr>
<td>0.994**</td>
<td>0.940**</td>
<td>0.999**</td>
<td>0.977**</td>
</tr>
<tr>
<td>0.979**</td>
<td>0.991**</td>
<td>0.997**</td>
<td>0.966**</td>
</tr>
</tbody>
</table>

**Correlation is significant at the 0.01 level (2-tailed).


DETERMINATION OF MAJOR SUGARS IN FRESH AND DRIED SPICES AND VEGETABLES USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Liga Priecina, Daina Karklina

Department of Food Technology, Faculty of Food Technology, Latvia University of Agriculture, Liela iela 2, Jelgava, Latvia, e-mail: liga.priecina@gmail.com

Abstract

Carbohydrates are one of the most important energy sources in plants synthesized during photosynthesis. They are important for plants to grow and produce. Polysaccharide content changes depending on plant type, growing location and weather conditions. The highest content differs in roots and leaves- photosynthesis starts in leaves, but to grow and produce mostly carbohydrates are localized in roots. It is important to follow up to differences in sugars changes in vegetable and spices. There are many researches focusing on sugar changes mostly in fruits and cereals, less in vegetables, herbs, and spices. The aim of this research was to determine major sugars in nine spices and vegetables- celery (Apium graveolens var. dulce), parsley (Petroselinum crispum), dill (Anethum graveolens), leek (Allium ampeloprasum), garlic (Allium sativum), onion (Allium cepa), celery root (Apium graveolens var. rapaceum), pumpkin (Curcubica maxima), carrot (Daucus carota) grown in Latvia in 2013. Analyses were made using high performance liquid chromatography (HPLC) in the laboratories of Latvia University of Agriculture, Faculty of Food Technology for fresh samples and samples dried in convective dryer at 45±1 °C temperature. Fructose, glucose, sucrose and maltose content was determined using HPLC method, and moisture content in each sample was established by drying samples till constant weight. Results were expressed as grams per 100 grams of dry weight (DW). Analysing obtained results the highest total sugar content was found in vegetable roots (carrot), lowest- in spices’ leaves. Convective drying at 45±1 °C process takes from 12 till 48 hours to reduce moisture content. After drying process in most cases individual sugar content increased significantly. During drying process water is removed from samples, sugars are changing, which may indicate possible Maillard reaction.

Keywords: spices, vegetables, drying, sugars, HPLC.

Introduction

By culinary definition vegetables are edible plant parts including stems and stalks, roots, tubers, bulbs, leaves, flowers, some fruits and seeds (Pennington, Fisher, 2009). Vegetables are an important part of our diet. They provide not only the major dietary fibre component in our food, but also a range of micronutrients, including minerals, vitamins and antioxidant compounds, such as carotenoids and polyphenols (Singh et al., 2012). Most vegetables are commonly cooked before being consumed. Cooking induces significant changes in chemical composition, affecting the bio accessibility and the concentration of nutrients and health-promoting compounds (Mazzeo et al., 2011).

Health beneficial attributes of spices have the potential of a possible therapeutically exploitation in a variety of disease conditions. Spices do not contribute significantly to the nutrient makeup of food, especially because of their small quantities encountered in diet (Srinivasan, 2005). Spices can be added to foods in several forms: as whole spices, as ground spices, or as isolates from their extracts. Spices are aromatic and pungent food ingredients (Suhaj, 2006).

Carbohydrates are divided into following groups- monosaccharides (simple sugars that are the simplest members of carbohydrates, cannot be subjected to hydrolysis); oligosaccharides (consists of small number of monosaccharides, usually two to ten molecules, with disaccharides being the most common in foods; polysaccharides (consists of large amount of monosaccharides). They vary in their physical and chemical properties compared to monosaccharides. Most important polysaccharides in food are starch, glycogen and cellulose (Tzia et al., 2012). The final products of carbohydrate digestion in the digestive tract are almost entirely glucose, fructose, and galactose. Because glucose is the only carbohydrate that can be oxidized in muscle, much of the fructose and all of galactose are transported to the liver, after absorption from the intestinal tract, and converted into glucose. The conversion of fructose and galactose occurs in the liver at relatively low rates (Campbell, 2013). Simple monosaccharides such as glucose can be directly absorbed by the host. Disaccharides such as maltose, lactose and sucrose can be hydrolyzed to their respective monosaccharides, but the ability to digest complex plant polysaccharides such as inulin, pectin and xylan is very limited (Ibrahim, Anishetty, 2012). One of the oldest methods for food preservation is drying, which consists of removing water from the product in order to provide microbiological safety and the most popular drying method is air-drying in convective drier. In this method the drying agent supplies heat to the material and removes moisture from material at the same time (Nawirska et al., 2009). Major disadvantages of hot air drying of foods are low energy efficiency and long drying time during the falling rate period. Because of the low thermal conductivity of food materials in this period, heat transfers to the inner sections of foods during conventional heating is limited (Wang, Xi, 2005). The drying time of the convective technique can be shortened using higher temperatures which increase moisture diffusivity and by cutting the material into smaller pieces. Increased drying temperature entails higher costs and may cause biochemical changes that degrade the dried product quality (Figiel, 2009).
Materials and Methods

All samples were grown and harvested reaching full maturity in Latvia in 2013. Analyses were made in the laboratories of Latvia University of Agriculture, Faculty of Food Technology.

Fresh samples were washed and cut in equal small pieces. For drying samples were washed, cut in small pieces and dried in a convective dryer with fan (Memmert, Model 100-800) at 45±1 °C temperature till constant (not changing) mass.

For samples moisture content was determined according to AOAC (1995). Samples were dried at 105±1 °C: fresh samples for 2 hours and dried samples for 30 minutes. Analysis were triplicated. Moisture content was expressed as percentes. From moisture was calculated dry matter.

Sugars content (fructose, glucose, sucrose and maltose) was determined using high performance liquid chromatography (HPLC) method (Shimadzu LC 20 Prominence) (Rybak-Chmielewska, 2007; Dimins et al., 2008; Beitane et al., 2013). Method is based on chromatographic separation of sugars and their retention time. Sample extracts were made to extract sugars according to following procedure: samples were soaked in water and extracted in ultrasound bath (SELECTA P ULTRASONS) at 35±1 °C for 45 minutes. Solution was filtrated twice using filter paper and 0.45 µm Millipore membrane.

The analysis by HPLC were performed at 25±1 °C under isocratic conditions. The mobile phase consisted from A – acetonitrile; B – water (70:30). Flow rate was 1.00 mL min⁻¹ and injection volume was 10 µL. The analytic column Alltech-NH₂ (4.6 mm×250 mm×5 µm) was used and refractive index detector (RID-10A) was used. Total time of analysis was up to 30 min. The identification of sugars in vegetables and spices was done by comparing retention times of individual sugars in the reference vs. tested solution (qualitative analysis). The quantitative assays were made by the following carbohydrates: fructose, glucose, sucrose and maltose. The content of those compounds were assessed based on the comparing peak areas obtained from the reference analysis. Analysis was done duplicate. Results were expressed as grams per 100 g of dry weight (g 100 g⁻¹ DW).

Results and Discussion

The highest dry matter content among fresh samples was established in garlic, lowest – in onion. In dried samples dry matter content was higher than in fresh samples (Table 1).

### Table 1

<table>
<thead>
<tr>
<th>Samples</th>
<th>Dry matter in fresh samples, %</th>
<th>Dry matter in dried samples, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Celery</td>
<td>17.57±0.18</td>
<td>90.66±0.91</td>
</tr>
<tr>
<td>Parsley</td>
<td>25.30±0.25</td>
<td>91.22±0.91</td>
</tr>
<tr>
<td>Dill</td>
<td>18.40±0.18</td>
<td>93.04±0.93</td>
</tr>
<tr>
<td>Leek</td>
<td>11.07±0.11</td>
<td>87.33±0.87</td>
</tr>
<tr>
<td>Onion</td>
<td>6.20±0.06</td>
<td>78.92±0.79</td>
</tr>
<tr>
<td>Garlic</td>
<td>45.59±0.46</td>
<td>87.44±0.87</td>
</tr>
<tr>
<td>Celery root</td>
<td>12.96±0.13</td>
<td>90.46±0.90</td>
</tr>
<tr>
<td>Carrot</td>
<td>11.60±0.12</td>
<td>84.87±0.85</td>
</tr>
<tr>
<td>Pumpkin</td>
<td>8.21±0.08</td>
<td>89.14±0.89</td>
</tr>
</tbody>
</table>

Dried onion, leek, garlic, carrot and pumpkin are samples which can absorb water from air in short time during grinding process. Fructose and glucose content increased after drying in all samples (Table 2). All results are expressed as grams per 100 g dried weight (g 100 g⁻¹ DW). Increasing can be explained with polysaccharide reducing in smaller monomers. Vegetables and spices contain a large amount of poly carbohydrates. The highest fructose content was determined in dried onions, lowest in fresh garlics. After drying process in most cases fructose content increases significantly (p<0.05). Only pumpkins fructose content decreases. The highest glucose content was reached in dried celery roots, lowest was in fresh garlics. Glucose content in most cases increases significant (p<0.05). Glucose content in pumpkins decreases significant (p<0.05).

Comparing reached data to USDA Nutrient database, glucose content in carrots are 0.28 g 100 g⁻¹ fresh weight (FW), in onions – 1.97 g 100 g⁻¹ FW, in celery 0.55 g 100 g⁻¹ FW, in dried parsley 2.76 g 100 g⁻¹ DW, in dried onion 0.73 g 100 g⁻¹ DW, in dried garlic 0.07 g 100 g⁻¹ DW. Fructose content in carrots are 0.26 g 100 g⁻¹ FW, in onions 1.26 g 100 g⁻¹ FW, in...
Fructose and glucose content in fresh and dried vegetables and spices

Table 2

<table>
<thead>
<tr>
<th>Samples</th>
<th>Fructose, g 100 g⁻¹ DW</th>
<th>Glucose, g 100 g⁻¹ DW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Celery</td>
<td>1.035±0.104</td>
<td>4.619±0.462</td>
</tr>
<tr>
<td></td>
<td>1.806±0.181</td>
<td>12.407±1.241</td>
</tr>
<tr>
<td>Parsley</td>
<td>3.527±0.353</td>
<td>9.219±0.922</td>
</tr>
<tr>
<td></td>
<td>5.437±0.544</td>
<td>11.729±1.173</td>
</tr>
<tr>
<td>Dill</td>
<td>1.089±0.109</td>
<td>2.282±0.228</td>
</tr>
<tr>
<td></td>
<td>3.245±0.325</td>
<td>4.41±0.441</td>
</tr>
<tr>
<td>Leek</td>
<td>12.647±1.265</td>
<td>10.838±1.084</td>
</tr>
<tr>
<td></td>
<td>12.232±1.223</td>
<td>13.322±1.332</td>
</tr>
<tr>
<td>Onion</td>
<td>18.442±1.844</td>
<td>18.094±1.809</td>
</tr>
<tr>
<td></td>
<td>26.891±2.689</td>
<td>23.209±2.321</td>
</tr>
<tr>
<td>Garlic</td>
<td>0.273±0.027</td>
<td>0.097±0.001</td>
</tr>
<tr>
<td></td>
<td>1.143±0.011</td>
<td>0.317±0.032</td>
</tr>
<tr>
<td>Celery root</td>
<td>0.815±0.082</td>
<td>20.586±2.059</td>
</tr>
<tr>
<td></td>
<td>1.200±0.120</td>
<td>25.059±2.506</td>
</tr>
<tr>
<td>Carrot</td>
<td>10.345±1.035</td>
<td>13.161±1.316</td>
</tr>
<tr>
<td></td>
<td>14.135±0.141</td>
<td>24.559±1.456</td>
</tr>
<tr>
<td>Pumpkin</td>
<td>18.867±1.887</td>
<td>17.771±1.777</td>
</tr>
<tr>
<td></td>
<td>13.492±1.349</td>
<td>12.837±0.128</td>
</tr>
</tbody>
</table>

A-fresh samples; B-dried samples

The highest sucrose content was determined in dried celery roots, the lowest in fresh dill. The highest maltose content was in dried pumpkins, but not detected in fresh carrots (Table 3).

The highest sucrose content was determined in vegetable roots, less in leaves. Maltose content has no differences between vegetable roots and vegetable leaves in most cases. Content of sucrose and maltose increases significantly (p<0.05) after drying.

Comparing reached data to USDA Nutrition database, sucrose content in carrots are 1.92 g 100 g⁻¹ FW, in onions 0.99 g 100 g⁻¹ FW, in celery 0.11 g 100 g⁻¹ FW, in dried parsley 4.09 g 100 g⁻¹ DW, in dried onion 3.87 g 100 g⁻¹ DW, in dried garlic 2.05 g 100 g⁻¹ DW. Maltose content in all previously described has not been detected. According to Caruso et al. (2014), sucrose content in onions is 8.32 g 100 g⁻¹ DW, in carrots 20.09 g 100 g⁻¹ DW. According to Caruso et al. (2014), sucrose content in onions is 11.3 g 100 g⁻¹ DW. Maltose content was not analysed in both researches. Obtained results can be compared with literature data.

Increasing monosaccharide and disaccharides could be explained by samples possible oligosaccharides division in more simple compounds. According to Yang et al. (2014), the pre-treatment and hydrolysis of cellulosides and oligosaccharides can be generated from hydrothermal (steaming, blanching, sterilization, drying etc.) pre-treatment of the cellulose. Using hydrolysis of oligosaccharides fermentable sugars can be recovered. Hydrolysis can be done using acid, alkaline, enzymes and oxidative degradation.

In future research will be analysed polysaccharide changes in fresh and dried vegetables and spices and how they are associated with antioxidant enlargement or reducing.

Conclusions

During drying process fructose, glucose, sucrose and maltose content significantly increased. Increasing may be explained with oligosaccharide dividing in monomers and dimers or during possible Maillard reaction. Catalyst for the decomposition reaction is heat. Spices and vegetables contain lower sucrose and maltose content, but higher fructose and glucose content. Vegetables and spices can be used in diets with low calorie amount than fruits.
References


PORE SIZE DISTRIBUTION OF EGGPLANTS DRIED BY DIFFERENT DRYING METHODS

Ayca Aydogdu1,2, Gulum Sumnu1, Serpil Sahin1

1 Department of Food Engineering, Middle East Technical University, Ankara, Turkey, e-mail: serp@metu.edu.tr
2 Department of Food Engineering, University of Necmettin Erbakan, Konya, Turkey

Abstract

The main objective of this study is to investigate the effects of hot air drying and microwave-infrared combination drying on porous structure of eggplants. Hot air drying was performed in a tray dryer at 50°C with an air velocity of 1.5 m s⁻¹. In microwave-infrared combination oven, different microwave powers (30%, 40% and 50%) were combined with different infrared powers (10%, 20%). During drying process, initial moisture content decreased from 14 kg water kg⁻¹ dry solid to approximately 0.13 kg water kg⁻¹ dry solid for eggplants. Pore size distributions of dried samples were analyzed with mercury porosimetry. Pores in different samples were characterized by cumulative intrusion curves which showed total volume of mercury intruded the poresin any size range and the threshold diameter. Cumulative intrusion curves had a sharp rise that indicated existence of macro pores on the surface. Unlike microwave–infrared combination dried eggplants, hot air dried eggplants did not have pores above 200 µm size. Threshold diameter of eggplants dried in microwave-infrared combination oven were in the range of 48.86–73.42 µm which were greater than that of hot air dried eggplant, 42.47 µm. Microwave-infrared combination drying provided more porous structure than hot air dried ones due to higher internal pressure. As infrared and microwave power increased, threshold diameter increased and eggplants with more porous structure were obtained.

Keywords: microwave, infrared, drying, eggplant, porosity.

Introduction

Eggplant (Solanum melongena L.) is an important market vegetable of Asian and Mediterranean countries. It contains a variety of phytochemicals such as phenolics and flavonoids (Akanitapichat et al., 2010). It is ranked amongst the top ten vegetables in terms of antioxidant capacity due to the phenolic constituents (Cao et al., 1996). However, due to the higher moisture content, they have limited shelf life. In order to extend their shelf lives, it is common to dry eggplants. Dried eggplants can be used as an ingredient in different kinds of meals, instant soups and sauces.

Drying is a removal of moisture from the food materials to prevent the growth and reproduction of spoilage microorganisms and to slow down the action of enzymes and minimize many of the moisture mediated deteriorative reactions (Wu et al., 2007). Texture, appearance, colour, flavor, taste and nutritional value are affected from drying process conditions. Therefore, deciding suitable drying system is very important for food products. Although hot air drying method is used most commonly in industry, during hot air drying, food is exposed to heat for longer time. It causes problems related to quality parameters such as unacceptable color, flavor, texture, sensory characteristics, loss of nutrients, shrinkage, reduction in bulk density and rehydration capacity (Maskan, 2001). In order to eliminate these problems, microwave heating can be used for drying of foods. It provides high internal pressure that causes shorter drying time and more porous structure. In recent years, microwave drying has been used in drying of carrots (Arikan., 2012), potatoes (Wang et al., 2004) and apples (Askari et al., 2006). However, due to the lower surrounding temperature, microwave heating causes condensation at the food surface. In order to eliminate this problem, Datta, Ni (2002) recommended the combination of infrared heating with microwave heating to dry food products. Infrared heating increases the surface temperature so excess surface moisture evaporates. Therefore, microwave-infrared combination drying has time saving advantage without the formation of sogginess. However, in literature, there are limited studies on microwave-infrared combination drying (Sumnu et al., 2005; Tireki et al., 2006). Sumnu et al. (2005) dried carrot by using microwave-infrared combination and hot air drying. It was stated that by using microwave-infrared combination drying, drying time was reduced to 98% of hot air drying time. Moreover, in the case of microwave-infrared combination drying, less color change and higher rehydration capacity of carrots were obtained as compared to conventional drying. Tireki et al. (2006) produced bread crumbs by conventional and microwave-infrared combination drying and it was stated that by using microwave-infrared combination drying, drying time was reduced to 96.8–98.6% of conventional drying.

One of the most important parameters that shows the effect of drying process on the quality of food is pore structure. More porous structure is an indication of less damage during drying. However, there is limited information about pore structure of dried fruits and vegetables. Schiffmann (1986) and Krokida, Maroulis (1999) analyzed porosity of microwave-dried apples, bananas, carrots and potatoes and concluded that microwave drying increased product porosity. Russo (2013) studied the effect of air temperature on microstructure of hot air dried eggplants and stated that the porosity increased with the air temperature. However, the effect of microwave-infrared combination drying on pore structure of eggplants has not been studied yet. Therefore, the objective of this study was to compare the effects of hot-air conventional drying and microwave-infrared combination drying on pore structure of eggplants.
Materials and Methods

Material

Eggplants (Solanum melongena L.) used in this study were obtained from the local market and stored in a refrigerator at 4°C. Prior to drying, samples were washed and cut into slices, of a thickness 5 mm using a kitchen slicer. Diameters of the eggplants were 5.0±0.5 cm and the weight of each sample was 7.0±0.5 g. The initial moisture content of eggplant was found to be 14±0.314 kg water kg⁻¹ dry solid by using moisture analyzer (MX-50 AND Moisture Analyzer, Tokyo, Japan).

Drying method

Hot air drying was performed in a tray dryer (Armfield Limited, D 27412, Ringwood Hampshire, England). The size of the tray was 18×30 cm². Drying experiments were carried out at 50°C with an air velocity of 1.5 m s⁻¹ until a moisture content of 0.13±0.002 kg water kg⁻¹ dry solid was reached in almost 4 hours. In each experiment, 100–115 g of eggplants were dried and weight of samples was recorded at every 1 hour interval. That is, 1.85–2.13 kg eggplants were placed on one m² of tray for drying. Microwave-infrared combination drying experiments were performed in microwave-infrared combination oven (Advantium oven™, General Electric Company, Louisville, KY, USA). In the experiments, powers of halogen lamps two at the top and one at the bottom which are sources of infrared power were 1500 W. Combinations of different microwave power levels (30%, 40% and 50%) and infrared powers (10% and 20%) were used in drying of eggplants. The power of the oven was determined as 630 W by using IMPI 2 liter test (Buffler, 1993). For both cases, 100–115 g of eggplant was dried until a final moisture content of approximately 0.13 kg water kg⁻¹ dry solid was achieved in almost between 19–30 minutes. In every 2 minutes, weight of the samples was recorded by a digital balance (ARD-110 - Single Unit, China).

Pore-size distribution

Pore size distribution was determined by using mercury porosimeter (Poremaster 60, Quantichrome Corp., Boynton Beach, Florida, USA). About 0.5 g of dried eggplants was used for each experiment. Measurements were done at pressure range of 0–50 psia, and for calculations, surface tension and contact angle of mercury was taken as 480 erg cm⁻² and mercury contact angle as 140°, respectively. Relation between applied pressure (P) and pore size was expressed by Washburn equation (Russo et al., 2013) which describes a linear relationship between the size of an intrudable circular pore and the applied mercury pressure in the mercury porosimeter.

\[ P.r = -2\gamma\cos\theta \]

where \( r \) is the pore radius (\( \mu m \)), \( \gamma \) is the Hg surface tension (N/m), \( \theta \) is the contact angle (°) and \( P \) is the absolute applied pressure (N).

Results and Discussion

The plot of cumulative volume of mercury intruded versus pore size or versus pressure is called as cumulative intrusion curve. From the cumulative intrusion curve, the total volume of mercury intruded the pore volume in any pore size range and the threshold diameter (the diameter above which comparatively low mercury intruded) can be determined (Aligizaki, 2006).

The cumulative volumes of mercury intruded as a function of pore size and pressure of dried eggplants using different combination of microwave and infrared powers were indicated through Figures 1 to 4. Figure 5 was given to show the cumulative intrusion curve of eggplant dried by hot air.

Initially, there was a sharp increase in intrusion volume and then a relatively constant region with increasing pressure in all graphs. Initial step rise indicated that there were macro pores on the surface and the same sized pores existed predominantly. This can be the result of less collapse in the structure due to short drying time. A gradual rise on the slope can be an indication of decreasing pore size through the sample (Rahman et al., 2002).

Pore size ranges for eggplants dried at 10% infrared and 30% microwave power, 20% infrared and 30% microwave power, 10% infrared and 50% microwave power and 20% infrared & 50% microwave power combinations were from 227.4 to 4.39 μm; from 201.3 to 4.28 μm; from 235.3 to 4.27 μm; 210.6 to 4.27 μm, respectively (Figures 1–4). When hot air dried eggplants were compared with microwave-infrared combined drying dried eggplants, pore size range of hot air dried eggplants was narrower. Unlike microwave-infrared combination drying eggplants, hot air dried eggplants did not have pores above 200 μm size (Figure 5).

Threshold pore size was the pore diameter where the vertical line was observed and the diameter above comparatively low mercury intruded. Threshold pore size of hot air dried eggplants was 42.47 μm which was lower than the threshold pore size of eggplants dried with microwave-infrared combination. This result could be related to higher shrinkage and lower porosity due to higher drying time of conventionally dried eggplants.

As infrared and microwave power increased, threshold pore size increased. Threshold pore sizes were 48.86 μm and 55.40μm for 10% infrared and 30% microwave power combination and 20% infrared and 30% microwave power combination, respectively. Also, the threshold pore sizes were 64.89 μm and 73.42 μm for 10% infrared and 50% microwave power combination and 20% infrared and 50% microwave power combinations, respectively. This can be attributed to the reduction of shrinkage due to the increased microwave and infrared power. Similar pattern was observed for spouted bed drying of wheat (Kahyaoglu, 2009). As the temperature increased, shrinkage was enhanced so threshold pore size decreased slightly.
Figure 1. Cumulative intrusion curve for eggplants dried in microwave-infrared combination oven at 10% infrared and 30% microwave powers

Figure 2. Cumulative intrusion curve for eggplants dried in microwave-infrared combination oven at 20% infrared and 30% microwave powers

Figure 3. Cumulative intrusion curve for eggplants dried in microwave-infrared combination oven at 10% infrared and 50% microwave powers

Figure 4. Cumulative intrusion curve for eggplants dried in microwave-infrared combination oven at 20% infrared and 50% microwave powers

Figure 5. Cumulative intrusion curve for eggplants dried in hot air dryer

Conclusions
According to cumulative intrusion curves, hot air dried eggplants had less porous structure than the ones dried in microwave-infrared combination oven. In addition, increasing microwave and infrared power resulted in more porous structure. The optimum drying condition is 20% infrared power and 50% microwave power combination that provides more porous structure. Thus, microwave-infrared combination drying can be recommended to be used for drying of eggplants.

References


CAROTENOIDS AND TOTAL PHENOLIC CONTENT IN POTATOES WITH DIFFERENT FLESH COLOUR

Irisa Murniece¹, Lolita Tomsonė¹, Ilze Skrabule², Aija Vaivode²

¹ Department of Food Technology, Faculty of Food Technology, Latvia University of Agriculture, 2 Liela Street, Jelgava, e-mail: irisa.murniece@llu.lv
² State Priekuli Plant Breeding Institute, 2 Zinatnes Street, Priekuli, Latvia

Abstract

Polyphenols are recognized as the most abundant antioxidants in our diet. Potatoes are a good source of these compounds. Phenolic compounds represent a large group of minor chemical constituents in potatoes, which play an important role in determining their organoleptic properties. Further, phenolics have a wide-array of health providing characteristics. The aim of this research was to determine the content of total phenolic and carotenoid (TPC) content in relationship with the colour of organically and conventionally cultivated potato varieties (Solanum tuberosum L.) with different flesh colour. In cooperation with the State Priekuli Plant Breeding Institute (Latvia), sixteen potato genotypes were studied. The highest TPC and carotenoid content was determined in potatoes of Purple Peru variety when conventionally cultivated. Correlation was found between TPC and colour L* (r=−0.813). The changes in carotenoid content and TPC of potatoes vary significantly according to the type of cultivation practise, depending on variety. There are common tendencies in the changes of TPC and carotenoid content – the variety was the most significant factor (p<0.001).

Keywords: potato, colour, TPC, carotenoids, organic and conventional.

Introduction

The research in potato chemistry has established the fact that there is a lot more in potatoes than starch. Natural colorant and antioxidant present in yellow-, purple- and red-flesh potatoes can be used for developing functional foods. Considering the large quantities in which potatoes are consumed throughout the world, potatoes could be a very good vehicle for addressing some health related problems (Ezekiel et al., 2013). Many of the compounds present in potatoes are important because of their beneficial effects on health, therefore, they are highly desirable in the human diet (Katan, De Roos, 2004).

Colour is an important food quality parameter. It affects consumer acceptance and can even evoke emotional feelings in humans (Crisosto et al., 2003; Ou et al., 2004). Coloured potatoes have attracted the attention of investigators as well as consumers because of their antioxidant activities, taste and appearance (Jansen, Flamme, 2006). The antioxidant activity in coloured potatoes is associated with the presence of polyphenols anthocyanins, flavonoids, carotenoids, ascorbic acid, tocopherols, alpha-lipoic acid and selenium (Lachman et al., 2005a). Therefore, coloured potatoes have the potential to be one of the richest sources of antioxidants in the human diet.

As food and life style choices have been increasingly recognised as useful approaches in prevention or delaying the onset of chronic diseases, more and more research and commercial development are focused on food phytochemicals such as polyphenolics and carotenoids (Maiani et al., 2009; Spencer, 2008; Minich, Bland, 2008; Hunter et al., 2008; Stevenson, Hurst, 2007).

Carotenoids are lipophilic compounds synthesized in plastids from isoprenoids which are widespread in nature and have broad range of functions, especially in relation to human health and their role as biological antioxidants (Dellapenna, Pogson, 2006; Fraser, Bramley, 2004). Because of their high carotenoids content potatoes are particularly beneficial for eye health (Tan et al., 2008).

Potato cultivars with white flesh contain less carotenoids as compared to cultivars with yellow or orange flesh. Total carotenoids content was reported in the range of 50–350 μg 100 g−1 FW and 800–2000 μg 100 g−1 FW, respectively, in white- and yellow-fleshed potato cultivars (Brown, 2008).

Polyphenols comprise over 8000 identified substances, which can be divided into groups according to their chemical structure, such as phenolic acids, stilbenes, coumarins, lignins and flavonoids (Ross, Kasum, 2002).

Phenolic compounds are considered to be health-promoting phytochemicals as they have shown in vitro antioxidant activity and have been reported to exhibit beneficial antibacterial, anticylgemc, antiviral, anticarcinogenic, anti-inflammatory and vasodilatory properties (Duthie et al., 2000; Mattilla, Hellstrom, 2006). Potatoes are a good source of phenolic compounds. Phenolic compounds represent a large group of minor chemical constituents in potatoes, which play an important role in determining their organoleptic properties. Further, phenolics have a wide-array of health providing characteristics (Bravo, 1998), therefore, have potential for use as functional food for improving human health. The phenolic content of potatoes was reported to be high, and ranged from 530 to 1770 μg g−1 (Al-Saikhan et al., 1995). Potatoes were considered the third most important source of phenols after apples and oranges (Chun, 2005).

Potato quality varies depending on the growing area, cultivar and aspects of the chemical composition of main crop potato tubers have been shown to depend on the cultivation system as well. The improved qualitative value of organic vs. conventional produce, however, has not been ascertained (Dangour et al.,
The interest in organic agriculture and environmentally-friendly agricultural products is increasing, and in particular consumers have made potatoes one of their top organic purchases among fresh vegetables even though organic potatoes carry a price significantly higher than most other vegetables (Carillo et al., 2012). In this respect, it is not known whether and how different agriculture techniques and/or cultivation systems may affect the nutrients composition of the final product. Comparison of organic and conventional foods in terms of nutritional value, sensorial quality and food safety, has often highlighted controversial results. As a consequence, a clear link between cultivation system and nutritional profile of agricultural products is still missing (Bourn, Prescott, 2002). The aim of this research was to determine the content of total phenolic and carotenoid content in relationship with the colour of organically and conventionally cultivated potato varieties (Solanum tuberosum L.) with different flesh colour.

**Materials and Methods**

The potatoes were planted in the middle of May and harvested in last decades of August or first days of September. Field trials were conducted in three replications. The certified potato seed material was used. Seed tubers were planted in rows; the distance between rows was 0.7 m and the distance between tubers 0.3 m.

**Organic field.** The soil type was sod podzolic (PVv), loamy sand. Organic matter content in soil was 25 mg kg$^{-1}$, pH$_{KCl}$ was 6.3, the availability in soil of K was low and P was medium. The common agronomic practices were used during vegetation period.

**Conventional field.** The soil type in conventional field was sod-podzolic (PVv), sandy loam. Organic matter content in soil was 27 mg kg$^{-1}$, pH$_{KCl}$ was 5.7, availability of K and P in soil was high. Fertilizer P – 55, K – 90 kg ha$^{-1}$ and N – 60 kg ha$^{-1}$ was used in conventional field. The common agronomic practices were used during vegetation period. Herbicides in field were used for weed control. The fungicides for restriction fungal diseases were used two times in July. The haulm was cut in last decade of August and the tubers were harvested in the beginning of September. Potatoes were stored at the State Priekuli Plant Breeding Institute at an air temperature of 4±1 °C and at a relative air humidity of 80±5%.

**Plant material.** In the experiment 16 potato (Solanum tuberosum L.) varieties with white, yellow and purple coloured flesh were evaluated, whose seed was obtained in the State Priekuli Plant Breeding Institute (SPPBI) (Latvia). Potatoes were grown in organic and conventional field and controlled by SPPBI. The characterization of potato varieties and the type of cultivation practice used per each potato variety is presented in Table 1.

### Table 1

<table>
<thead>
<tr>
<th>Name and acronym* of potato variety</th>
<th>Flesh colour</th>
<th>Origin of variety</th>
<th>Cultivation practice applied**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agrie Dzeltenie (Adz)</td>
<td>yellow</td>
<td>Latvia</td>
<td>O/C</td>
</tr>
<tr>
<td>Prelma (P)</td>
<td>yellow</td>
<td>Latvia</td>
<td>O/C</td>
</tr>
<tr>
<td>Imanta (I)</td>
<td>white</td>
<td>Latvia</td>
<td>O/C</td>
</tr>
<tr>
<td>Lena (L)</td>
<td>yellow</td>
<td>Latvia</td>
<td>O/C</td>
</tr>
<tr>
<td>Brasla (Br)</td>
<td>yellow</td>
<td>Latvia</td>
<td>O/C</td>
</tr>
<tr>
<td>Bionica (Bi)</td>
<td>white</td>
<td>Netherlands</td>
<td>O/C</td>
</tr>
<tr>
<td>Anuschka (An)</td>
<td>yellow</td>
<td>Germany</td>
<td>O/C</td>
</tr>
<tr>
<td>Gundega (G)</td>
<td>yellow</td>
<td>Latvia</td>
<td>O/C</td>
</tr>
<tr>
<td>S04009-37 (S37)</td>
<td>yellow</td>
<td>Latvia</td>
<td>C</td>
</tr>
<tr>
<td>S03135-10 (S10)</td>
<td>white</td>
<td>Latvia</td>
<td>C</td>
</tr>
<tr>
<td>S99108-8 (S8)</td>
<td>light</td>
<td>yellow</td>
<td>C</td>
</tr>
<tr>
<td>Fenton (F)</td>
<td>purple</td>
<td>US</td>
<td>C</td>
</tr>
<tr>
<td>Purple Fiesta (PF)</td>
<td>purple</td>
<td>US</td>
<td>C</td>
</tr>
<tr>
<td>British Columbia Blue (BCB)</td>
<td>purple</td>
<td>US/Canada</td>
<td>C</td>
</tr>
<tr>
<td>Purple Peru (PP)</td>
<td>purple</td>
<td>Peru</td>
<td>C</td>
</tr>
<tr>
<td>Blue Congo (BC)</td>
<td>purple</td>
<td>Czech Republic</td>
<td>C</td>
</tr>
</tbody>
</table>

* Acronyms of potato varieties used throughout the paper presented in brackets
** Cultivation practice: O/C – cultivated organically and conventionally, C – cultivated conventionally

**Sampling.** For testing, a total of 10 kg (around 50–60 potato tubers) of table potato tubers per variety were selected into small piles, from ten different wooden boxes. Five potatoes were selected from several location points of each box (Murniece et al., 2011). All operations during sample preparation were performed very quickly so as to avoid deviations from the qualitatively obtained results. Potato tubers were peeled and analysed without a skin. In the analysis on total phenolic content (TPC) and carotenoids, the test and analysis were run in triplicate and averaged.

**Carotenoids** were analyzed by spectrophotometric method (with the UV/VIS spectrophotometer Jenway 6705) at 440 nm (Ермаков, 1987) and is expressed on mg 100 g$^{-1}$ fresh weight (FW) of potatoes.

**Total Phenolic Content (TPC).** For extraction of phenolic compounds five grams of the homogenized sample were extracted with 50 mL of ethanol water solution (80%) in a conical flask with a magnetic stirrer (magnet 4.0×0.5 cm) at 700 rpm for 1 h at room temperature (20±1 °C). The potatoes extracts were then filtered via the paper with No 89. The TPC of the extracts was determined according to the Folin-Ciocalteu spectrophotometric method.
(Singleton et al., 1999) with some modifications. To 0.5 mL of extract, 2.5 mL of Folin-Ciocalteu reagent (diluted 10 times with water) and, after 3 minutes 2 mL of sodium carbonate (Na₂CO₃) (75 g l⁻¹) was added. The sample was mixed. After 30 minutes of incubation at room temperature, the absorbance was measured at 765 nm. Total phenols were expressed as gallic acid equivalents (GAE) mg 100 g⁻¹ FW of potatoes.

**Colour analysis.** The colour of potato samples was measured by “Colour Tec-PCM” device. For evaluation of the colour of potato samples, potato slices were cut shortly before measurement in order to avoid formation of melanin pigments during non-enzymatic browning reaction which can affect the accuracy of colour measurement. Potato samples were covered by a transparent PP film (“Forpus”), thickness of 25 μm, to avoid direct contact between the equipment of the measuring device and the product. The colour was measured at least in seven various locations of the sample in order to obtain higher accuracy after calculation of the mean value. For data processing, “ColorSof QCW” software was used.

**Statistical Analysis.** For statistical analysis, the data were processed using the S-PLUS 6.1 Professional Edition software and XLSTAT 2014 program. Data are presented as a mean ± standard deviation (SD). The differences between independent groups were specified by one way and two way analysis of variance (ANOVA), and values of p<0.05 were regarded as statistically significant. In case of establishing statistically significant differences, homogeneous groups were determined by Tukey’s multiple comparison test at the level of confidence α=0.05. Relationships between carotenoid, total phenolic content and colour were made by Principal Component Analysis (PCA).

**Results and Discussion**

Carotenoid content in potatoes cultivated organically varied from 0.089 to 0.385 mg 100 g⁻¹ FW while in conventionally cultivated potatoes – from 0.068 to 0.371 mg 100 g⁻¹ FW (Fig. 1). Bonierbale et al. (2009) has found that carotenoid content might vary in potatoes from 0.103 to 2.135 mg 100 g⁻¹ FW). The results of current research make the range much wider. Differences in this case might be influenced by several factors, for example variety and maturity stage of tubers (Murniece et al., 2011). It has been found that total carotenoid content is higher in immature tubers and it decreased with tuber maturity (Kotikova et al., 2007; Morris et al., 2004).

Comparing coarotenoid content between potato tuber varieties with white and yellow flesh, content was found to be the highest in Lenora variety when cultivated organically and Anuschka variety when cultivated conventionally.

In potato tubers with purple flesh carotenoid content was 78% lower than in potato tubers with white and yellow flesh and it varied from 0.012 to 0.084 mg 100 g⁻¹ FW (Table 2).

In the evaluation of carotenoid content within the factor of variety and factor of cultivation practise, statistical results showed that significance was noticed between varieties (p=0.019) while no significant difference was noticed when potato tubers were cultivated organically or conventionally (p=0.316). The most significant difference in carotenoid content was determined between Imanta and Anuschka varieties.

Kotikova et al. (2007) have obtained the same result that the application of fertilizers did not bring any significant changes in carotenoids of potatoes. The TPC might be affected during the development of the flesh colour (purple, violet, yellow) of potato tubers (Lachman et al., 2005b), due to the environmental conditions, such as longer days and cooler temperatures or fertilization (Kumar et al., 2004; Reyes et al., 2004).

TPC in potato tubers cultivated organically varied from 34.475 to 64.230 mg GAE 100 g⁻¹ FW, in 25% of analysed potato tubers TPC was no higher than 36.937 mg GAE 100 g⁻¹ FW while in 75% of analysed potato tubers TPC reached to 49.436 mg GAE 100 g⁻¹ FW. In the case when potato tubers were cultivated conventionally TPC was in lower amount and it varied from 26.854 to 52.172 mg GAE 100 g⁻¹ FW. In 25% of analysed potato tubers TPC was no more than 31.410 mg GAE 100 g⁻¹ FW of while in 75% – 49.040 mg 100 g⁻¹ FW (Fig. 2).

Comparing the obtained results with Faller and Failho (2009), also in this particular research, TPC significantly differed between varieties (p=0.015) while cultivation practise did not present significance (p=0.164). The most significant difference in TPC was determined for Anuschka variety when compared with Brasla, Imanta and Lenora varieties.
Comparing TPC of potatoes with white and yellow flesh with purple fleshed it was 81% higher in potato tubers with purple flesh. The highest TPC between purple fleshed varieties was determined for Purple Peru variety while the lowest — in Purple Fiesta variety (Table 2).

Table 2

<table>
<thead>
<tr>
<th>Name of potato variety</th>
<th>Carotenoids</th>
<th>TPC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg 100 g(^{-1}) FW</td>
<td>mg GAE 100 g(^{-1}) FW</td>
</tr>
<tr>
<td>Fenton</td>
<td>0.012±0.002</td>
<td>75.92±0.14</td>
</tr>
<tr>
<td>Purple Fiesta</td>
<td>0.070±0.002</td>
<td>69.54±0.56</td>
</tr>
<tr>
<td>British Colombia Blue</td>
<td>0.084±0.005</td>
<td>73.02±0.38</td>
</tr>
<tr>
<td>Purple Peru</td>
<td>0.029±0.002</td>
<td>86.72±0.25</td>
</tr>
<tr>
<td>Blue Congo</td>
<td>0.030±0.001</td>
<td>77.07±0.23</td>
</tr>
</tbody>
</table>

Results present means of triplicate determinations with ± standard deviation

To better understand the relationship between TPC and carotenoid content with colour of organically and conventionally cultivated potato varieties, PCA was applied. In Figure 3 the first and second principal components accounted for 73.47% of total variance (PC1 – 51.22% and PC2 – 22.25%). Projection of the samples in the space formed by PC1 and PC2 shows that colour intensity (L*) is low of potato tuber flesh of Imanta variety and for the same (i.e. Imanta variety) the difference in flesh colour was noticed when potatoes were cultivated organically and conventionally (Fig. 3). In addition, the projection of PCA presents the opposite side of potato varieties with yellowish flesh (b*) and with higher carotenoid content (carotenoids) of potato varieties projected in the same area. Potato varieties projected in the positive area of F1 and F2 are higher in TPC and colour flesh is much lighter than in the varieties projected in the positive area of F1 and negative of F2 i.e. of b* and carotenoids.

When potatoes of purple flesh were analysed by applying PCA, the projection of factors was different from potatoes with white and yellow flesh. In the Figure 4 first and second principal components accounted 86.58% of total variance (PC1 – 55.51% and PC2 – 31.07%). Potato varieties which are projected in the negative area of F1 are higher in TPC and colour flesh is much darker (purplish to bluish) whereas in the positive area of F2 with the predominant factor L* Blue Congo variety cultivated conventionally comparing to other potatoes tubers with purple flesh was with lighter colour (Fig. 4).

Figure 2. Total phenolic content in potato tubers with white and yellow flesh

Figure 3. Principal component analysis, projection of potato varieties with white and yellow flesh cultivated organically and conventionally

The first letter indicates variety and the second – cultivation practice. An example: I_O – Imanta variety cultivated organically while L_C – Imanta variety cultivated conventionally. Acronyms are summarized in the Table 1.

Figure 4. Principal component analysis, projection of potato varieties with purple flesh cultivated conventionally

First letter indicates variety and second – cultivation practise. An example: BC_C – Blue Congo variety cultivated conventionally. Acronyms are summarized in the Table 1.
In the correlation analysis (Fig. 5) of potato tubers with white and yellow flesh obtained results represented negative correlation between TPC with the colour of redness value a* \((r=0.594)\), positive correlation was found between TPC and carotenoid content with the colour of yellowness value b* \((r=0.490)\) and \((r=0.591)\) respectively.

When correlation analysis was applied to all potato varieties used in the research, between TPC and \(L^*\) correlation was found \((r=0.813)\).

In the colour analysis the obtained results showed that colour intensity within potato tuber varieties with white and yellow flesh significantly differed between Imanta and Prelma varieties \((p<0.05)\). Significant difference was noticed on yellowness value \(b^*\) \((p<0.001)\) while factor \(a^*\) which represents redness did not show significance on colour flesh between all potato varieties with white and yellow flesh \((p>0.05)\). In the case of statistical analysis of potato tubers with purple flesh there were no significant affect on any of parameters determined in research \((p>0.05)\).

**Conclusions**

From the nutritional point of view, apart from the macronutrient content, potatoes contain traces of nutrients which are proved to be beneficial for human health. From the results of this particular research it might be concluded that the colour of potato flesh could be one of the factors taken into account for modelling and predicting health promoting substances like carotenoid, anthocyanin content and also TPC. In future, much deeper investigation is required in this particular field of interest.

**Acknowledgment**

The authors acknowledge financial support from the following: project „Sustainable use of local agricultural resources for development of high nutritive value food products (Food)” within the National Research Programme “Sustainable use of local resources (earth, food, and transport) – new products and technologies (NatRes)”.

**References**


THE INFLUENCE OF PRE-TREATMENT METHOD ON THE FAT CONTENT DECREASE IN FRENCH FRIES
Solvita Kampuse, Aleksandrs Jefimovs, Tatjana Rakcejeva

Abstract
French fries are very popular product in many countries. But this product together with potato chips is included in the group of unhealthy products due to high content of fats, acrylamid, and peroxide value. Therefore the aim of this research was to evaluate the pre-treatment methods for reducing the fat content, and improving other quality parameters.

Before frying the potato strips were blanched in hot water at 85 °C, and dried in microwave-vacuum dryer for 3, 6 and 8 min, and in conventional dryer with air ventilation for 15 min at 60 and 80 °C, and 10 min at 100 °C temperature. The frying was done at 170 °C for 1.5–2 min. The colour (in CIE L* a* b*), texture (with texture analyser), the moisture content, the total fat content, and the ascorbic acid content were analysed for all prepared samples.

The best preliminary drying technology was drying in the conventional dryer at 100 °C temperature for 10 min. The product prepared with this technology had the lowest total fat content (3.4%) and the lowest hardness (lowest maximal cutting force: 11.3 N). The lowest moisture content was to sample dried in microwave-vacuum dryer for 8 min (36.84%). This sample was also the hardest one (maximal cutting force: 52.41 N). There were no significant differences (p>0.05) in colour (L* a* b*) values between control samples and samples dried in conventional dryer but the samples dried in microwave-vacuum dryer became darker than control samples (lower L* value 57.37).

Keywords: French fries, preliminary drying, total fat content.

Introduction
French fries are very popular product in many countries. But this product together with potato chips is included in the group of unhealthy products due to high content of fats, acrylamid, and peroxide value.

High oil content is a major factor affecting consumer acceptance of oil-fried products today and the low fat food products are becoming more popular (Bunger, Moyano, Rioseco, 2003; Mai Tran et al., 2006). Oil consumption especially saturated fat is considered a major factor increasing health risks such as coronary heart disease (CHD), cancer, diabetes and hypertension, and even linked to increased causes of deaths. Fried foods contribute a significant proportion of the total fat consumed in the Western world. The fat content in French fries produced by different companies can vary between 3.4 and 20.9% (USDA National Nutrient Database for Standard Reference). That proves the influence of production technology and other factors to the total fat content of this product.

Knowledge of optimal frying conditions is important to produce French fries with low fat content, both under industrial conditions and at home.

During deep-fat frying, water in the crust will evaporate and move out of the food. In order for the flow of vapour to continue, sufficient water has to be able to migrate from the core of the food to the crust and the crust has to remain permeable. The fact that the vapour leaves voids for the fat to enter later, is the reason why fat uptake is largely determined by the moisture content of the food (Mehta, Swinburn, 2001). Similarly, sections of the food with more moisture loss also show higher uptake of fat. Some even argue that the total volume of fat will equal the total volume of water removed (mass balance) (Pinthus et al., 1993).

An increase in the density of potato tuber also leads to a decrease in fat absorption. In one study of fried potato chips, tubers with a higher density (1.103 g cm\(^{-3}\)) yielded chips with lower fat content (42.1%) than slices with lower density of 1.093 g cm\(^{-3}\) (48.8% fat) (Ulheil, Escher, 1996). The thickness of the potato strips is an important factor affecting the overall fat content of French fries. Thick-cut strips (12 mm or bigger) absorb less fat than thin-cut strips (Blumenthal, 1991).

In the preparation of French fries, several processes are involved. The most common sequence is cutting – blanching – drying – pre-frying – freezing / chilling – packing – thawing. Each step may be important for the final product quality. The fat uptake varies with the pre-treatment of the potato. Blanching reduces the subsequent cooking time. Blanching also makes the colour more uniform after frying and it forms a layer of gelatinized starch that limits oil absorption and improves texture (Moreira, 1999).

Low temperature blanching enhances pectin methyl esterase (PME) activity and is another option proposed that affects both textural quality and oil uptake (Aguilar et al., 1999). Drying the surface of the potato strips before frying reduces the fat uptake in French fries. In the experiments of Lambeg et al. (1990) drying reduced the fat uptake by 7 to 29% (for 1 min frying) and by 15 to 40% (for 5 min frying). Drying causes a ‘skin’ to form on the surface of the potato strip and this reduces vapour transport through the surface layer. Raising the temperature of the fat higher than 185 °C causes the fat to break down more rapidly due to an increased rate of oxidation and polymer formation in the fat. When frying at 200 °C or above, excess energy in the fat is converted into cross-links leading to case-hardening of food. This results in a brown surface forming on the food before the inside is completely cooked and immersing the food for a longer time to cook the inside properly may cause burning on...
the outside (Blumenthal, Stier, 1991). In some countries maximum frying temperature is set at 180 °C. On the other hand, if French fries are cooked at lower temperature, or the ‘boiling action’ on the surface ceases due to a lower heat input, the crust does not form on the surface. This allows extra fat to penetrate into the core of the French fries. About 40% more fat is absorbed when the fat temperature is 10 °C lower than the recommended cooking temperature of 180 °C to 185 °C (Mehta, Swinburn, 2001).

The reducing of fat content in fried products is became as very actual topic of the research but there is still not a uniform opinion on the best solutions for reducing the fat content in fried French fries. There is a little information also about the influence of different pre-drying methods to the possible of the reducing of oil content in French fries. Therefore the aim of this research was to evaluate the pre-treatment methods for reducing the fat content, and improving other quality parameters.

Materials and Methods

The experiments were carried out in the laboratories of the Faculty of Food Technology, Latvia University of Agriculture. The potatoes of cultivar ‘Laura’ were used. The samples were cut into straws manually by hands 5×5 mm wide. Before frying the potato straws were blanched in hot water at 85 °C for 8 min, and dried in microwave-vacuum dryer MYCCOH-1 for 3, 6 and 8 min, and in conventional dryer with air ventilation for 15 min at 80 °C, and 10 min at 100 °C temperature. The frying was done at 170 °C for 2 min. The colour (in CIE L*a*b*), texture (with texture analyser), the moisture content, the total fat content, and the ascorbic acid content were analysed for all prepared samples.

Determination of textural properties

The textural properties of potato straws were measured in terms of the cutting force. A Texture Analyzer TA.XTPlus (Stable Microsystems, UK) was used for cutting force determination. HDF/BSw blade set with Warner Blaxter was used for detection of cutting force to potato straws, moving at a test speed of 1.5 mm s⁻¹ over a distance of 5.0 mm. The numerical results were expressed in N.

Total fat content

Determination of total fat content was occurred by using the SoxCapTM2047 in combination with Soxtec extraction systems. The total fat content was tested to samples, which were treated with oil, and with oil and water, but was not tested to samples treated only with water because of too low fat content.

Colour measurements

Colour of samples was measured in CIE L*a*b* colour system using Tristimulus Colorimeter, measuring Hunter colour parameters by Colour Tec PCM/PSM. Colour values were recorded as L* (brightness) – the vertical co-ordinate runs from L* = 0 (black) through grey to L* = 100 (white); a* (-a, greenness, +a, redness) – the horizontal co-ordinate, that runs from -a* (green) through grey to +a* (red) and b* (-b, blueness, +b, yellowness) – another horizontal co-ordinate, that runs from -b* (blue) through grey to +b* (yellow) (Couloute, 2002). The measurements were repeated on different randomly selected locations at the surface of each sample.

Total colour difference (ΔE*) between control sample and samples dried in different conditions was calculated using the following equation (1):

\[ \Delta E^* = \sqrt{(L^*-L_0^*)^2 + (a^*-a_0^*)^2 + (b^*-b_0^*)^2} \]  

(1)

where ΔE* – total colour difference; L*, a* and b* are the lightness (L), greenness (a) and blueness (b) values for the pre-dried samples; L₀, a₀ and b₀ are the corresponding colour values for the control sample. The difference L*-L₀ is difference of lightness, a*-a₀ – difference of green and red colour and b*-b₀ – difference of blue and yellow colour.

The moisture content

Moisture content was determined using standard method ISO 6496:1999 by verified balance KERN (Germany) with precision ±0.001 g; mass loss calculation (%) were determined by weighing samples on the electronic scales, by standard LVS ISO 1442:1997.

The content of ascorbic acid

The content of ascorbic acid was determined by titration with 0.05-M iodine solution (Jansons, 2006). The French fries sample (25 g) were poured with 6% solution of oxalic acid and homogenized. Then the sample was filtered. 2 mL of 1% solution of starch was added to 10 mL of filtrate and the filtrate was titrated until the colour changed which does not disappeared during a 30 sec interval. For standard solution of ascorbic acid 20 mg of ascorbic acid were dissolved in 100 mL of the oxalic acid solution. Two mL of the starch solution was added to 25 mL of the standard-solution and the mixture was titrated. The content of vitamin C (ascorbic acid) mg per 100 g of the product dry matter was calculated with the following equation (2):

\[ C = \frac{5000 \cdot \frac{V_{\text{sample}}}{m \cdot V_{\text{standard}}}}{g} \]  

(2)

where \( V_{\text{sample}} \) – volume of the iodine solution titrated in a sample, mL; \( V_{\text{standard}} \) – volume of the iodine solution titrated in a standard solution, mL; \( m \) – the amount of sample, g.

Statistical analysis

The results were processed by mathematical and statistical methods. One-way analyses of variance (p≤0.05) were used to determine significance of differences between samples prepared by different drying methods in the main quality parameters: hardness, total fat content, colour (L*, a*, b*) and ascorbic acid content.
Results and Discussion

Textural properties

There were significant differences (p<0.001) in maximal cutting force between potato samples prepared with different pre-treatment methods. The samples dried in conventional dryer were with more similar textural properties comparing to control sample than samples dried in microwave-vacuum dryer (Fig. 1).

Figure 1. The texture of French fries pre-treated with different methods

The highest maximal cutting force was to French fries sample dried before frying in microwave-vacuum dryer for 8 minutes (in average 52.13 N) but the lowest maximal cutting force was observed to sample dried in conventional dryer at 100 °C temperature.

Total fat content

As the aim of these investigations was to find the method for production of French fries with the reduced oil content then total fat content of samples is very important quality parameter. The total fat content of control sample (French fries blanched at 85 °C for 7 min, and just fried without drying) was in average 10.15% but in all samples, which were dried after blanching, the fat content was significantly lower (p=0.028) and didn’t reach 8% (Fig. 2).

Figure 2. The total fat content of French fries pre-treated with different methods

The lowest total fat content was detected in samples dried in conventional dryer: in average 4.10% in samples dried at 80 °C, and 3.40% in samples dried at 100 °C. The samples dried in microwave-vacuum dryer contained higher amount of oil than conventionally dried samples. The effect of pre-drying to the oil content of French fries was found also by some other authors (Krokida et al., 2001). According to the results of these authors pre-drying pre-treatment had a significant effect on oil uptake and moisture loss, as well as structural properties of French fries. The use of air drying caused decreasing of the oil and moisture contents of French fries, while the porosity increased.

The colour L* a* b* of different French fries samples

Colour is another important factor in the qualified processing of French fries. The brown colour of potato chips is formed by a reaction between reducing sugars and amino acids. Brown colour of potato chips is formed when the moisture content decreases to below 6–12% (Mai Tran et al., 2006). Similarly the brown colour in French fries starts to form after rapid decreasing of moisture content in outside layers of potato strips. There were no significant differences found between samples in colour value a* (p=0.057), and value b* (p=0.338) but the brightness (L* value) significantly differed between samples (p<0.001) (Fig. 3).

Figure 3. The brightness (L* value) of French fries pre-treated with different methods

The brightest was the sample dried at 80 °C temperature in conventional dryer (L*=69.01) but the darkest was the sample dried in microwave-vacuum dryer 8 min (L*=57.37). In comparison with control sample the lowest total colour difference ∆E was observed also to sample dried at 80 °C temperature in conventional dryer but the highest colour difference ∆E was to sample dried in microwave-vacuum dryer 8 min (Fig. 4). Drying in conventional dryer does not change the colour of French fries while after drying in microwave-vacuum dryer the colour becomes darker. It could be explained with faster decreasing of moisture from the inside cells of potato strips. The authors Song et al., 2007 in the experiments with vacuum-fried potato chips with microwave-vacuum pre-drying also found that the vacuum-microwave pre-drying had a negative effect on colour of potato chips, which decreased the L* value of potato chips and increased Hunter a* and b* values.


The moisture content

The moisture content of all French fries samples was similar, and there were no significant differences (p=0.21) found between samples pre-treated with various methods. But still there was tendency that samples dried in microwave-vacuum dryer for longer time contained lower moisture content than control sample.

The ascorbic acid content

The ascorbic acid content significantly differed between French fries samples (p=0.007). The highest ascorbic acid content was detected in sample dried in microwave-vacuum dryer at 100 °C (in average 50.7 mg 100 g\(^{-1}\) of dry weight). The higher ascorbic acid content in microwave-vacuum dried potato products was detected also by other authors (Khraisheh, 2004). Our investigations with different fruit and vegetables showed higher retention of ascorbic acid after microwave-vacuum drying process compared to conventional air drying, too (Galoburda et al., 2012; Sne, Kampuse, 2011; Dorofejeva et al., 2011). The sample with the lowest ascorbic acid content was French fries dried in conventional dryer at 100 °C (in average 38.09 mg 100 g\(^{-1}\) of dry weight) (Fig. 5).

With lowering the drying temperature in conventional dryer it was possible to save higher amounts of ascorbic acid – the French fries dried at 80 °C contained in average 47.56 mg 100 g\(^{-1}\) of dry weight ascorbic acid. The influence of temperature to the content of ascorbic acid in conventionally dried cranberries was proved also by investigations of Dorofejeva et al., (2010) where ascorbic acid content in cranberries rapidly decreased by rising temperature. But in experiments with red pepper scientists found that both vitamin C content and the total phenolic content decreased as air-drying temperature decreased (Vega-Gálvez et al., 2009). It means that processes of vitamin C degradation during drying can be influenced by the type of product and many other factors.

Conclusions

The drying in conventional dryer provided better quality parameters in the end product than drying in microwave-vacuum dryer. The lowest total fat content in French fries can be reached by drying in conventional dryer at 100 °C, but the lowest colour difference ΔE and the most similar cutting force to the control sample can be provided by drying in conventional dryer at 80 °C temperature.

Acknowledgment

This publication has been prepared within the State Research Programme 2010.10-4/VPP-5/4 (VP27) “Sustainable use of local resources (earth, food, and transport) – new products and technologies (NatRes)” (2010–2013.) Project no. 3. “Sustainable use of local agricultural resources for development of high nutritive value food products (Food)”. 

References


PACKAGING MATERIAL AND STORAGE-INDUCED QUALITY CHANGES IN FLEXIBLE RETORT POUCH POTATOES’ PRODUCE

Aija Ruzake, Sandra Muizniece-Brasava, Lija Dukaliska

Department of Food Technology, Faculty of Food Technology, Latvia University of Agriculture, 2 Liela str., Jelgava, Latvia, e-mail: aija.ruzaike@gmail.com

Abstract

Experiments have been carried out at the Latvia University of Agriculture Department of Food Technology. The aim of this work was to appraise the quality changes of potato produce in dry butter and mushroom dressing which was thermally treated in vacuum packed soft retort pouches and stored for 12 months at ambient temperature ±18°C. A retort pouch is a heat-resistant bag made of laminated plastic films or foil. It is then heat-sealed and sterilized by pressure cooking in a retort. Two laminated polymers with barrier properties were used: transparent polyamide/polyethylene (PA/PE) and lightproof polyethylene terephthalate/aluminium/polyamide/polypropylene (PET/ALU/PA/PP). Samples of potato products in packaging were analysed immediately after retort thermal treatment and after 1, 2, 3, 4, 6, 8, 10 and 12 months of storage. The quality changes of potato produce during storage was characterized by measuring such parameters as structure, pH, colour and microbiological parameters. Data obtained emphasize the importance of selected laminate PET/ALU/PA/PP with aluminium layer preventing light transparency which could provide stable quality of ready to eat potato produce thermally treated in retort pouch for long-duration up to 12 months. The aim of this work was to appraise the quality changes of potato produce in dry butter and mushroom dressing which was thermally treated in vacuum packed soft retort pouches and stored for 12 months at ambient temperature ±18±2°C.

Keywords: retort pouch, quality, storage, potatoes.

Introduction

Nowadays, potatoes are popular for human consumption around the world and in more than 100 countries potatoes are staple food for many (Singh, Kaur, 2009). Raw potatoes are difficult to digest, therefore several types of preparation, such as cooking, baking, braising, grilling, and microwave processing, are used to improve the structure of potatoes and make them more accessible to the diet (Carini et al., 2013). The main factors that affect potato quality among consumers are potato size, colour and texture after cooking. When purchasing processed potato products the focus is on the appearance, taste, texture and nutritional value of the product (Suryawanshi, 2008). Today, food market offers a wide range of potato varieties; most of them are classified according to organoleptic characteristics, quality and end-use (Lopez-Vizcón, Ortega, 2012). The demand for vacuum-packed, healthy, cut potatoes is ever increasing (Lante, Zocca, 2010). With increasing consumer demand for convenience and high quality products, potato processing companies constantly look for new and innovative ways to use potatoes effectively (Abu-Ghannam, Crowley, 2006). Popular processed potato products include potato crisps, French fries (chips), potato powder, potato cubes, slices and potato starch (Suryawanshi, 2008). During the recent years, development of new food products has been receiving increased attention. It is mainly because the demand for safe food with extended shelf life has been increasing in both consumer and merchant groups (Valceschini, 2006). Heat treatment is a common food preservation method. Based on the intensity of the treatment, it can be divided into two main groups – pasteurization and sterilization. Each of these treatments has its own specific features (intensity, suitability for the type of product), which determined the shelf life of food. Heat treatment can also affect the quality of food. The optimal treatment regime needs to be selected for each type of food in order to minimize unwanted quality losses and increase the desired quality, while ensuring microbiological safety (Lammens et al., 2013). Sterilization is one of the most important processes to maintain packaged food quality (Marra, Romano, 2003). Currently, treatment of products in packaging is one of the leading processing technologies that impart long-term storage at room temperatures (Ito et al., 2014).

Using this method, the product can maintain most of its original texture, as well as minimal loss of nutrients can be ensured, in addition to obtaining a longer shelf life. This technology provides a number of advantages; it allows to effectively managing the heat from the steam or water to the product through the packaging, preventing recontamination of the product. It also excludes off-flavour formation and oxidation of the product, preventing moisture and nutrient losses during evaporation and loss of volatile compounds during cooking, as well as preventing development of aerobic microorganisms in the product. Heat treatment of vegetables and fruits in packing is recognized as an excellent way to maintain their structure without losing such valuable nutrients as vitamins and minerals, which can significantly decrease when vegetables and fruits are boiled in water. This high nutrient retention contributes to the persistence of flavour and colour in such vegetables as turnips, swedes, carrots etc. (Sansone et al., 2012).

Packaging is essential for products intended for long-term storage because storage time and temperature significantly affect the appearance, aroma, taste and structure (Clark et al., 2002). Thermal treatment of food products in soft packaging is becoming more widely used and it serves as an alternative to metal cans. Packaging bags intended for heat treatment usually consist of up to three layers: polypropylene (PP), aluminium foil and polyethylene.
Terephthalate (PET). Aluminium foil and polyethylene terephthalate are primarily used to provide the desired barrier properties. Polypropylene provides an excellent sealing of packaging. Polypropylene layer protects aluminium foil from the inside and promotes overall strength (Byun et al., 2010, Srinivasapop Gopal et al., 2001).

Suitable and qualitative packaging has a major role in maintaining the quality of the product and also in the convenience of customer use (Alvenainen, 2003). The quality of products must be maintained during storage; therefore food companies need to constantly work on improving recipes, readjusting packaging materials and technology in order to reduce the initial product quality changes during storage. In turn, scientists need to look for more innovative ideas to provide entrepreneur, producer, and customer satisfaction (Puligundla et al., 2012).

Materials and Methods

Research was carried out during the period from November 2011 to January 2014. Product samples were prepared at vegetable processing company in Latvia. Physical and chemical analysis (colour, hardness, pH) were performed at the Packaging material property testing laboratory at the Department of Food Technology (Latvia University of Agriculture). Microbiological and nutrition analyses and corresponding to them expiry date were defined at the laboratory of Food and Environmental Investigations at Institute of Food Safety, Animal Health and Environment BIOR.

In total, three different types of samples were prepared: control sample of cut potatoes without dressing, and two samples of potatoes with butter and mushroom (Agaricus bisporus) dressing. Raw potatoes were peeled and cut into equal-sized 8 mm wide slices. After dressing addition, potato products were dosed in soft retort pouches made out of two different packaging materials, portion mass in each pouch was 500±0.05 g. Two laminated polymers with barrier properties were used: transparent polyamide/polyethylene (PA/PE) and lightproof polyethylene terephthalate/aluminium/polyamide/polypropylene (PET/ALU/PA/PP) films. Size of each pouch was 200×250 mm, thickness of film 80 μm. Potato products were placed in soft retort pouches, vacuum-sealed using a two-chamber vacuum packaging machine Falcon 2-70; hermetic sealing mode – vacuum, 20 MPa, sealing time 3.8 s for PE/PA pouches and 5.3 s for PET/ALU/PA/PP pouches. A retort pouch is a hermetically sealed bag made of laminated plastic films or foil. It is then heat-sealed and sterilized by pressure cooking in a retort "Lagarde", sterilizing temperature +121±2 °C, cooling temperature +20±2 °C. Samples were stored for 12 months at ambient temperature +18±2 °C. Physical and chemical properties as well as microbiological testing of products were carried out on the production day (day 0) and after 1, 2, 3, 4, 6, 8, 10 and 12 months of storage. Samples were encrypted with letters and numbers (Fig. 1), samples in PE/PA packaging were marked as P1 (control sample), P2 (sample with butter dressing) and P3 (sample with mushroom dressing), while samples in PET/ALU/PA/PP packaging were marked as A1 (control sample), A2 (sample with butter dressing) and A3 (sample with mushroom dressing).

Colour analysis

Colour of potato product samples were determined using Colour Tec PCM / PSM with CIE L*a*b* colour system. Measurements were completed in tenfold repetition for each sample for more precise calculations of the mean value and standard deviation. Measurements were recorded using data program Colour Soft QCW. Total colour difference (ΔE*) of potatoes’ products between initial value and after storage was calculated using the following equation 1: (MacDougall, 2002).

\[
ΔE^* = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2}
\]

where ΔE* – total colour difference of the product during storage, L* – colour intensity value at the final product storage day, L0* – colour intensity value at day 0, a* – value of colour component green – red at the final product storage day, a0* – value of colour component green – red at day 0, b* – value of colour component blue – yellow at the final product storage day, b0* – value of colour component blue – yellow at day 0.

Determination of nutritional value

Nutritional value of developed products was determined using the following methods: protein content (BS EN 12135:2001 standard), carbohydrates were determined by difference, fat content (GOST 8756.21-89 p.2 method), crude fibre content (ISO 5498:1981), ash content (GOST 25555.4-91 p.2 method), moisture content (GOST 28561-90 p.2
method). Energy value was calculated according to the results of analysis above. Nutritional values testing of products were carried out on the production day.

**Determination of hardness**

The hardness of potato products was determined with TA.XT. Plus Texture Analyser, Blade set with knife (Stable Microsystems, UK). Data collection and analysis was carried out with program Texture EXPONENT 32. Blade movement speed during the test mode was 1 mm s⁻¹ (forwards) and 10 mm s⁻¹ (backwards). The force at which data collecting started was 0.04903 N. Measurements were completed tenfold for each sample for more precise calculations of the mean value and standard deviation.

**Determination of pH**

pH of potato products was determined using pH-meter JENWAY 3510 with electrodes JENWAY 3 mol / KCl (standard method ISO 1132:2001).

**Microbiological analysis**

Total plate count (TPC) was determined according to the standard LVS EN ISO 4833:2003. To determine sulphite-reducing *Clostridia* in the product (*Clostridium* spp.), GOST 29185-91*, standard method was used.

**Data analysis**

The obtained data were processed using SPSS 16.0 software package; arithmetic mean value and standard deviation were calculated. The results were analysed using univariate analysis of variance (ANOVA). Factors were rated as significant if p-values < α₀.₀₅. For the interpretation of the results it was assumed that α=0.05 with 95% confidence probability.

**Results and Discussion**

Nutrition is an important factor for consumers when making a food choice. More attention is paid to the composition of ingredients and nutritional value of products because the number of food-related diseases is ever increasing (Patras et al., 2009). Therefore, nutritional value of potato products was determined and the results are given in Table 1. The results show the energy value of control sample – 301 kJ 100 g⁻¹, which mainly constitutes of potato carbohydrates (15.7%), followed by protein (1.8%) and a small proportion of total fat (0.1%). Potatoes contain about 20% of dry matter; the rest is water (Ek et al., 2012). Total water content in the control sample is 81%. The addition of dressing mixtures to potatoes changes and affects their nutritional value. Nutrition changes are mainly related to the dressing mixture composition. Energy value of potatoes with butter dressing is 346 kJ 100 g⁻¹, which is by 45 kJ more than in the control sample. The sample with butter dressing contains 16.8% carbohydrates, which is 1.1% more than in the control sample. Potatoes with butter dressing contain about 0.2% more protein and 0.9% more fats than the control sample; moisture content is for 2% lower.

Energy value of potatoes with mushroom dressing is 341 kJ 100 g⁻¹. This sample contains fewer kJ than potatoes with butter dressing. Total carbohydrate content in potatoes with mushroom dressing is 17.7%, which is by 2.0% and 0.9% more than in the control sample and potatoes with butter dressing, respectively. Protein content in potatoes with mushroom dressing is 2.4%, which is by 0.6% higher and 0.4% lower than in the control sample and potatoes with butter dressing, respectively. Moisture content in potatoes with mushroom dressing is 78.2%, which is by 3.2% and 0.8% lower than in the control sample and potatoes with butter dressing, respectively. The results show that addition of dry dressing mixtures does not significantly change energy value of potato products.

**Table 1**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Content per 100 g of product</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control sample</td>
</tr>
<tr>
<td>Energy value</td>
<td>71 kcal / 301 kJ</td>
</tr>
<tr>
<td>Protein, %</td>
<td>1.8</td>
</tr>
<tr>
<td>Carbohydrates, %</td>
<td>15.7</td>
</tr>
<tr>
<td>Fat, %</td>
<td>0.1</td>
</tr>
<tr>
<td>Crude fibre, %</td>
<td>0.6</td>
</tr>
<tr>
<td>Moisture, %</td>
<td>81.0</td>
</tr>
<tr>
<td>Ash content, %</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Colour is one of the main factors which the consumer perceives as the quality indicator of the product (Suryawanshi, 2008). Generally, the colour may be affected by genetic factors and technological parameters of production process. Technological parameters include packaging material, light exposure during storage and the type of preparation (Murcia et al., 2003). Total colour changes of potato products during the storage are characterized by the total colour difference ΔE* which is calculated from measured L*, a* and b* values.

There are significant disparity (p<0.05) between the total colour difference ΔE* of potato products arising during storage in studied PE/PA pouches (p<0.05) and PET/ALU/PA/PP packaging (Fig. 2), which can be explained by light transmission of packaging material. After the comparison of the samples packaged and stored in both packaging materials for 12 months, it can be concluded that PET/ALU/PA/PP packaging ensures a significantly (p<0.05) lower product colour changes than PE/PA packaging.

There are significant disparity (p<0.05) between the total colour difference ΔE* of potato products arising during storage in studied PE/PA pouches (p<0.05) and PET/ALU/PA/PP packaging (Fig. 2), which can be explained by light transmission of packaging material. After the comparison of the samples packaged and
stored in both packaging materials for 12 months, it can be concluded that PET/ALU/PA/PP packaging ensures a significantly (p<0.05) lower product colour changes than PE/PA packaging.

Figure 2. The total colour difference ΔE* of potato products after 12 months of storage


An important quality indicator of thermally-treated potatoes in packaging is structure which indicates the hardness of the product; therefore changes in potato hardness after thermal treatment are mainly due to the changes in the structure and chemical composition (Abu-Ghannam, Crowley, 2006).

Figure 3. Changes in hardness of potato products during storage


In order to evaluate potato hardness during 12 month storage period, samples of potato products in both packaging materials, which were stored at a constant temperature +18±2 °C, were compared (Fig. 3). During the six month period of storage, hardness of potato products has risen evenly in both packaging materials from 8.5 to 10.8–11.8 N; during the first half of storage (6 months) the studied packaging materials evenly affected the changes in potato hardness. However, a significant increase in hardness (p<0.05) was observed after 12 months of storage in PE/PA packaged potato products from 11.8 to 15.6–19.2 N. These significant changes occurred because PE/PA packaging has higher water vapour permeability; hence it cannot maintain initial quality of potato products during storage. Changes in structure of heat-treated products in packaging are usually caused by water loss during storage (Alonzo et al., 2013).

pH is an important factor as it determines which microorganisms can proliferate in the product (Suryawanshi, 2008). Initial pH for potatoes without dressing was 5.84, for potato products with dressing – 5.75 (Fig. 4).

Figure 4. Changes in pH of potato products during storage


Stability in pH during storage of heat-treated products in packaging is an important quality indicator. Potato products in PE/PA packaging presented slight decrease in pH during storage; pH in the control sample reduced to 5.81, in the sample with butter dressing to 5.73 and in the sample with mushroom dressing till 5.75, these changes were not significant (p>0.05). Changes in pH of potato products in PET/ALU/PA/PP packaging were not significant (p>0.05) as well, while pH in the control sample rose to 5.86 and in samples with dressing pH rose or dropped to a small extent. It can be concluded that both PE/PA packaging and PET/ALU/PA/PP packaging are able to provide practically constant pH during storage. This is an important indicator when assessing the possible development of microorganisms in thermally treated products in packing during storage.

Consumer demand for food depends on the quality of the product, convenience of use, and safety of the product (Chen et al., 2012). One of the most common types of food contamination is biological
contamination. It is relevant to emphasize the importance microbiological contamination has on public health, because direct contact and other transfer factors help microorganisms to get and proliferate in a variety of foodstuffs and the digestive tract of animals and humans. Microbiological contamination of food is one of the most common types of food contamination not only in countries where there are problems with provision of food hygiene requirements, but also in the European Union countries and the United States where a high level of consumer protection is ensured (Food and Veterinary Service Research Centre, 2004).

Total plate count and the presence of sulphite-reducing Clostridia were determined immediately after thermal treatment and during 12 month storage period. The total plate count (number of microorganisms) in potato products in both types of packaging materials during storage did not exceed 10 CFU g⁻¹. It clearly demonstrates that the selected treatment regime and the choice of packaging material can ensure product safety.

Potatoes and potato products are recognized as one of the main foods that may cause a significant risk of becoming infected with *Clostridium* species bacteria (Barker et al., 2005). *Clostridium* species are anaerobic spore-producing bacteria, so it is necessary to ensure effective heat treatment process to eliminate the risk of infection. Sulphite-reducing *Clostridia* were not present in any of the samples during storage which indicates the effectiveness of the technology used and the ability to ensure the security and safety of the product during storage.

Microbiological indicators are one of the main criteria that determine shelf life of the product and its safe use in the diet. According to the results of the total plate count, absence of sulphite-reducing *Clostridia*, as well as such quality characteristics as colour, hardness and pH of potato products, the optimal shelf life for thermally treated potato products in PE/PA packing stored at the temperature of +18±2 °C could be three months but thermally treated potato products in PET/ALU/PA/PP pouches can be used up to 12 months.

**Conclusions**

Changes in the total colour difference ΔE* of potato products during 12 month storage packed in PET/ALU/PA/PP are not significant (p>0.05), while significant colour changes have been observed in potato products packed in PE/PA (p<0.05).

A steady increase in potato hardness from 8.5 N to 10.8–11.8 N was observed during the six month storage in both packaging materials. After 12 month storage, a significant (p<0.05) increase in hardness was observed in potato products stored in PE/PA packages (from 11.8 N to 15.6–19.2 N). The selected thermal treatment in vacuum packed soft retort pouches and both types of packaging materials provide microbiological safety of potato products during 12 month storage at +18±2 °C temperature, but only laminated lightproof PET/ALU/PA/PP film provides constant product quality during storage.

Potato products in PET/ALU/PA/PP packaging are safe for human consumption up to 12 months of storage.

**References**

PHYTASE ACTIVE YEASTS ISOLATED FROM BAKERY SOURDOUGHS

Lina Nuobariene, Nils Arneborg, Åse Solvej Hansen

Department of Food Science, Faculty of Science, University of Copenhagen, Rolighedsvej 30, DK-1958 Frederiksberg, Denmark, e-mail: aiva@food.ku.dk

Abstract

Nowadays, consumption of whole-grain bread is gaining popularity across the world due to its many health promoting effects. Despite that whole grains contain 20–70% of the daily requirements of the minerals, their absorption in human gut are very low due to antinutritional phytate. To increase the bioavailability of minerals, enzymatic degradation of phytate is needed. The aim of this study was to isolate and identify phytase active yeasts from sourdoughs. The use of selected microorganisms with high phytase activity could find use as starter cultures to improve mineral bioavailability in whole-grain bread fermented in a short time as alternative to sourdough bread. In addition, purification and characterization of phytase produced by Saccharomyces cerevisiae was done. The screening of phytase-positive strains was carried out at temperature of 30 °C and pH 5.5, conditions optimal for leavening of bread dough. To investigate whether the yeasts produce phytases selective defined minimal medium was developed. Phytase activity was determined colorimetrically by measuring the inorganic phosphate released by enzyme. Specific extracellular phytase activities of yeasts were at least 20-fold higher than the intracellular activities. The highest activities were observed in two Saccharomyces cerevisiae isolates, i.e. L1.12 (10.6 U (10^10 CFU)^-1) and L6.06 (8.2 U (10^10 CFU)^-1). The purified phytase produced by S. cerevisiae was most active at pH 4.0 and 35°C. This phytase is quite stable at pH range from 3.5 to 3.5 and temperature range from 25 °C to 40 °C.

Keywords: phytase, sourdough, yeast, Saccharomyces cerevisiae.

Introduction

Major epidemiological studies have shown that consumption of whole-grain foods is protective against the development of several chronic diseases. Whole-grains have been found to help regulation of blood glucose and protect against type 2 diabetes (de Munter et al., 2007) as well as reduce the risk of obesity (van de Vijver et al., 2009). The importance of whole-grain foods intake on blood glucose and weight regulation is considered as a result of the content of dietary fibre and magnesium in whole grains. Other studies concern the contribution of dietary fibre, folate and, to a certain extent, vitamin B₉ and magnesium from whole-grain foods as protective components against cardiovascular diseases and certain type of cancer (Slavin, 2004). Besides dietary fibre, the whole-grain foods are also important source of minerals. It was shown that 100 g of whole grains contain 20–70% of the daily requirements of the minerals, such as iron, zinc, magnesium, potassium, phosphorous and manganese (Cordain, 1999). However, most of the minerals in cereals are complexly bound to phytic acid as phytate, consequently reducing their bioavailability (Kumar et al., 2010).

Iron deficiency is the most common nutrient deficiency, widespread both in western and developing countries (WHO, 2002). Zinc represents the other nutritionally significant mineral that has been associated with phytate inhibition effect. To increase the bioavailability of minerals from a given food, enzymatic degradation of phytate and its dephosphorylated isomer IP₅ (inositol pentaphosphate) is needed (Sandberg et al., 1999). Efficient reduction of phytate can be achieved by enzymatic degradation during food processing, either by increasing the activity of endogenous phytase, or by addition of phytase-active yeast, lactic acid bacteria, or other microorganisms.

Cereals exhibit high phytase activities with values ranging from approx. 900 to 2900 U kg⁻¹ dry matter in wheat grain, and from 4100 to 6100 U kg⁻¹ dry matter in rye grain (Eeckhout, Depaepe, 1994; Greiner, Egli, 2003). However, the activities in wheat were considered insufficient to notably improve the mineral bioavailability in whole-grain wheat bread (Türk, Sandberg, 1992). Several studies were carried out on phytase activity from baker’s yeast (Andlid et al., 2004; Türk, Sandberg, 1992). They showed that only marginal amount of phytate was degraded during 3 h fermentation.

From an industrial point of view, the extracellular phytase activity would be more important for bread making than the intracellular phytase activity, because cells of the yeast should be intact in dough in order to ensure a good fermentation. In this case, the intracellular phytase will not have access to phytate in the dough. So far, no high phytase-active yeasts were identified for bread industry in order to increase mineral bioavailability.

The aim of this study was therefore to isolate and identify yeasts from different commercial sourdoughs and to study their phytase activity under conditions optimal for leavening of wheat bread dough (30 °C and pH 5.5) in order to identify species and/or strains with high phytase activities that might be used in baking industry. Further, purification and characterization of high-active extracellular phytase produced by generally recognized as safe (GRAS) yeast Saccharomyces cerevisiae was done.

Materials and Methods

Microorganisms. Yeasts tested for phytase activity were isolated from seven Lithuanian sourdoughs, further purified and identified (Nuobariene et al., 2012). Saccharomyces cerevisiae L1.12 isolated from rye sourdough and identified as a phytase-positive...
yeast strain was used for further phytase production and purification.

**Growth test for phytase-active yeasts.** To check the ability of yeasts to synthesize phytase, yeast strains were cultivated on Defined Minimal Medium (Delft) plates supplemented with phytic acid dipotassium salt (P5681, Sigma-Aldrich, Broendby, Denmark) as the only phosphorus source (Delft+Phy). As control, yeast strains were cultivated on phosphate-containing (Delft+P, positive control) and phosphate-free (Delft-P, negative control) medium (Nuobariene et al., 2012). Growth tests were performed both on solid and in liquid medium.

**Phytase assay.** Extracellular phytase extracts were prepared according to Nuobariene et al. (2011). Phytase activities were determined as described previously (Nuobariene et al., 2012). Briefly, 10 µl of enzyme extract was added into pre-incubated 40 µl 3 mM phytic acid dipotassium salt solution, gently mixed by inverting tube and incubated at 30 °C. Samples were taken every 15 min (0, 15, 30, and 45 min) during assaying. The reaction was stopped by adding 50 µl 10% TCA (trichloroacetic acid solution). A separate enzyme blank was prepared from sodium acetate/HCl buffer mixed with enzyme extract and TCA. Substrate blank was prepared from substrate solution mixed with TCA. To determine the content of liberated inorganic phosphate during phytase activity freshly prepared acyl molybdate solution was used in colorimetric analysis.

One unit of phytase activity was defined as the amount of phytase that liberates 1 µmol inorganic phosphate per minute from 3 mM phytic acid dipotassium salt at pH 5.5 and at 30 °C.

**Phytase purification.** The extracellular phytase produced by S. cerevisiae L1.12 was purified using ultrafiltration and affinity chromatography. All purification steps were performed at 4 °C.

After cultivation, the yeast cells from the fermented broth were removed by centrifugation (5000 g for 10 min, 4 °C); culture supernatant with extracellular protein fractions was collected and filtered (0.2 µm Minisart filters; Bie & Berntsen, Herlev, Denmark). 100 mL of prepared extracellular protein extract, as described above, was desalted and concentrated to 40 mL using Centriprep 3K centrifugal filter device with Ultracel YM membrane (the nominal molecular weight limit 3000).

The present of glycan on the yeast phytase protein (Seguelia et al., 1992) facilitated further purification of the desalted extract. Concanavalin A (Con A) sepharose, a lectin-conjugate resin that binds high glucose and mannose groups, was used for affinity chromatography. The sample was loaded at flow rate of 0.1 mL min⁻¹ onto Con A sepharose column (20 mm diameter×30 mm) equilibrated with buffer A (0.02 M Tris-HCl and 0.5 M NaCl, pH 7.2) and, afterwards, washed extensively (3 column volumes) to remove unbound proteins. Bound glycoproteins were competitively eluted with 0.25 M methyl-α-D-mannopyranoside solubilized in buffer A. Aliquots of eluent (1 mL) were applied to the column at interval of 30 min. Collected fractions (1 mL each) which show phytase activities were combined as a single fraction and assayed for phytase activity.

**Protein quantification.** Protein concentration was determined by measuring the absorbance of enzyme extract at 600 nm using the Bio-Rad Colorimetric Protein assay, Kit II (Bio-Rad Laboratories Inc). BSA (bovine serum albumin) was used as protein concentration standard.

**Effects of pH and temperature on phytase activity and stability.** The effect of pH on the phytase activity was determined by incubating the enzyme in phytic acid dipotassium solution at various pH (pH 3.0 to 7.0) using the standard assay conditions. The buffers used for reaction were 0.2 M sodium acetate / HCl (pH 3.0–6.0) or 0.2 M Tris-HCl (pH 6.0–7.0). The maximum activity was taken as 100% and percentage relative activity plotted against different pH values.

The effect of pH on phytase stability was studied by incubating the purified enzyme at 30°C for 1, 2 and 4 h at various pH values ranging from 3.0 to 7.0 followed by measuring the residual activity under standard assay conditions.

The phytase assays for determination of temperature optimum were performed as earlier outlined, except for the variation of the incubation temperature. For this purpose, phytase activity was assayed over a temperature range of 25 °C to 60 °C at 5 °C intervals. The maximum activity was taken as 100% and percentage relative activity were plotted against different temperatures.

Thermostability of the phytase was tested by incubating enzyme extract without substrate addition at a temperature range of 25 °C to 65 °C for 2 h and samples were taken every 10 min. Residual activities where then measured as indicated above, at a fixed temperature of 30 °C and pH 5.5.

**Results and Discussion**

**Phytase-active yeasts from sourdough.** The number of yeasts in all tested sourdoughs was about the same and ranged from 6.7 to 7.9 log CFU (colony forming units) g⁻¹ sample. In total, 140 yeast colonies were isolated, purified and subjected to the rep-PCR analysis. Based on results from cluster analysis, isolates were grouped according to their fingerprint pattern (Nuobariene et al., 2012). For a representative number of isolates within each group; i.e. 11 isolates in total, the identification to species level were further done by sequencing of the D1/D2-region of the 26S rRNA gene. Based on results from sequencing, two yeast species isolated from seven different sourdoughs were identified, i.e. *S. cerevisiae* and *Pichia kudriavzevii* (formerly named as Issatchenkia orientalis, anamorph Candida krusei). A pure culture of *S. cerevisiae* was found in five out of seven tested sourdoughs with fermentation temperature of 30–35 °C, while *P. kudriavzevii* was the dominant...
species in other two sourdoughs with fermentation temperature of 24–26 °C. To check the ability of identified 11 yeast strains to produce phytase they were cultivated on solid/liquid minimal Delft medium supplemented with phytic acid dipotassium salt as the only phosphorus source. All tested strains grew very well on Delft+Phy medium which indicates that tested strains produce phytase (Figure 1).

Figure 1. Optical density values of yeast cell growth at 30 °C in experimental media after 48 h incubation
Black bars – Delft-P medium, white bars – Delft+P medium, grey bars – Delft+Phy medium

However, from growth test, based on monitoring of cell growth in phytate rich environment, it is impossible to predict which yeast strains will have higher or lower activities (Nuobariene et al., 2011). Therefore, it is essential also to perform phytase activity assay on each strain.

The highest specific extracellular activity of all isolates was observed in S. cerevisiae L1.12 with a value of 10.6 U (10^{10} CFU)^{-1}, followed by S. cerevisiae L 6.06 with a value of 8.2 U (10^{10} CFU)^{-1} (Figure 2). Extracellular specific activities among other S. cerevisiae strains differed dramatically and ranged from 0.9 to 7.3 U (10^{10} CFU)^{-1}. The specific extracellular phytase activity values within three P. kudriavzevii strains varied between 1.1 and 4.6 U (10^{10} CFU)^{-1}.

Cultivation of S. cerevisiae L1.12 for phytase production. The activity of phytase in response to phytic acid dipotassium salt and the growth of S. cerevisiae L1.12 during 72 h of cultivation are shown in Figure 3. The yeast cells reached their stationary growth phase after 42 h of cultivation. Increase in phytase activity during the exponential growth phase was very slow.

However, rapid increase in phytase activity was observed in the late exponential growth phase (36–42 h of cultivation) and was continuously increasing during early stationary growth phase. It can be seen from Figure 3 that the maximum extracellular volumetric phytase activity (0.062±0.005 U mL^{-1}) was obtained when yeast cells reached the stationary phase and remained almost constant for the next 12 h of cultivation. Afterwards, slight decrease in phytase activity was observed in late stationary growth phase. This finding is in agreement with Quan et al. (2001) findings which showed that phytase production by Candida krusei occurred in the late stage of exponential growth phase and that phytase activity increased gradually with increasing incubation time.

Figure 2. Specific extracellular phytase activity from yeast strains of S. cerevisiae and P. kudriavzevii

Figure 3. Phytase activity and yeast growth at different time points
Circles – phytase activity, U mL^{-1}; triangles – yeast growth, ln (CFU mL^{-1})
Phytase purification. Up to now, there have been only few reports on the yeast extracellular phytases purification and enzymatic properties. During the last decade, extracellular phytases from yeast, including the wild strain of Schwanniomyces castellii (Segueilha et al., 1992), marine yeast Kodamaea ohmeri (Li et al., 2008), and S. cerevisiae isolated from the mash of traditional Korean wine (In et al., 2009) have been purified to homogeneity. However, phytase produced by yeast species, mentioned above mostly are attributed as potential for commercial application in the feed industry. Therefore, purification and characterization of S. cerevisiae L1.12 isolated from sourdough which may be an ideal candidate for improving mineral bioavailability in whole grain is of importance.

Table 1

<table>
<thead>
<tr>
<th>Purification steps</th>
<th>Total activity</th>
<th>Total protein</th>
<th>Specific activity</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture supernatant</td>
<td>24.6</td>
<td>4.62</td>
<td>5.3</td>
<td>100</td>
</tr>
<tr>
<td>Ultrafiltration</td>
<td>24.5</td>
<td>4.12</td>
<td>6.0</td>
<td>99.7</td>
</tr>
<tr>
<td>Con-A Sepharose</td>
<td>0.9</td>
<td>0.02</td>
<td>42.9</td>
<td>3.7</td>
</tr>
</tbody>
</table>

The results of phytase produced by S. cerevisiae L1.12 purification are summarized in table 1. The first purification step, ultrafiltration, leads to an increase in purity of only 1.1-fold but recover nearly all the protein from the original extract. Second purification step, a Con-A affinity chromatography, took advantage of the presence of high glucose and mannose side chains in phytase. This resulted in separation of phytase from major non-glycosylated proteins. The phytase was purified 8.1-fold, with a yield of 3.7% to a maximum specific activity of 42.9 U mg⁻¹.

Effect of pH and temperature on phytase activity. Figure 4 demonstrate the effect of pH variation on phytase activity produced by S. cerevisiae L1.12 at 30 °C (relative activities).

Figure 4. Effect of pH on the relative activity (solid line) and stability (dashed lines) of extracellular phytase from S. cerevisiae L1.12

The optimum pH at 4.0 for purified phytase is in agreement with Vohra and Satyanarayana (2001). In et al. (2009) reported that yeast phytase had sharp decline in activity as the pH value moved towards the neutral range 0.5-units from optimum. In contrast, we observed less than 15% decrease in relative phytase activity when the pH value was moved 0.5-units from optimum towards the neutral or acidic range. This can be explained by the fact that the pH of rye dough is about 4.3–4.6, except for the sourdough where pH is around 3.7 (Nielsen et al., 2007) and that phytase purified from rye sourdough yeast is well adapted for current conditions. However, in the whole wheat sourdough where pH varies between 5.3 and 5.5 the activity of yeast phytase seems to be only about 50–70% of optimum activity.

The effect of pH on the enzyme stability indicates that the extracellular phytase produced by S. cerevisiae isolated from rye sourdough is active in the pH range 3.5–5.5, with more than 80% of the initial activity remaining. However, sharp decline in activity was observed when the enzyme was incubated at pH 6.0 as well as in more neutral pH.

Figure 5 shows the effect of temperature variation on purified phytase activity at pH 5.5 (relative activities). Maximum purified extracellular phytase activity was exhibited at 35 °C with 98% and 78% of residual activity at temperature of 30 °C and 40°C, respectively. The residual activity at 55 °C was found to be only 7%. Comparison of the optimal temperature of phytase suggested that the phytase produced by S. cerevisiae L1.12 isolated from rye sourdough had slightly lower optimal temperature than those of S. cerevisiae isolated from the mash of traditional Korean yakju (40 °C) (In et al., 2009).

Figure 5. Effect of temperature on the relative activity of extracellular phytase from S. cerevisiae L1.12

Effect of temperature on the stability of purified phytase during different incubation time is summarized in table 2. During an incubation period of 2 h, no loss of phytase activity was observed at temperature below 40 °C, while about 8% of the activity was maintained after 15 min incubation at 55 °C. No phytase activity was detected when enzyme was incubated at 65 °C (Table 2). Although phytase produced by S. castellii and Pichia anomala have been reported to be
thermostable at temperature above 70 °C (Segueilhaet et al., 1992; Vohra, Satyanarayana, 2001), our findings are in agreement with those of Quan et al. (2002). They observed that phytase produced by C. krusei is easily inactivated at temperature above 50 °C. Further, our tested phytase at 45 °C displays an increasing trend in activity after 2 h of incubation. Even though the phytase of S. cerevisiae L1.12 has a low activity at 45 °C, seems that it is very stable at this temperature.

Table 2

<table>
<thead>
<tr>
<th>Temp, °C</th>
<th>Time in minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15</td>
</tr>
<tr>
<td>25</td>
<td>96</td>
</tr>
<tr>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>35</td>
<td>99</td>
</tr>
<tr>
<td>40</td>
<td>82</td>
</tr>
<tr>
<td>45</td>
<td>34</td>
</tr>
<tr>
<td>55</td>
<td>10</td>
</tr>
<tr>
<td>65</td>
<td>nd*</td>
</tr>
</tbody>
</table>

*nd – not detected

Conclusions

The highest extracellular phytase activities were observed in two S. cerevisiae strains; i.e. L1.12 and L6.06 and therefore they may be the best candidates for improving bioavailability in whole-grain bread.

Concanavalin A chromatography process was successfully implemented to purify phytase from S. cerevisiae L1.12 which resulted in 8.1-fold purification with a yield of 3.6% to a maximum activity of 42.9 U mg⁻¹. The optimum pH and temperature values for purified phytase were found to be 4.0 and 35 °C, respectively.

Acknowledgment

This project was financed be a PhD scholarship from faculty of LIFE Science, University of Copenhagen, Denmark. The technical assistance of Basheer Yousef Aideh is deeply appreciated.

References


FOODBALT 2014
THE INFLUENCE OF BAKING TEMPERATURE ON THE QUALITY OF TRITICALE BREAD

Martins Sabovics, Evita Straumite, Ruta Galoburda

Department of Food Technology, Faculty of Food Technology, Latvia University of Agriculture, Liela Street 2, Jelgava, Latvia, e-mail: martins.sabovics@inbox.lv

Abstract

During the baking, there occur different changes in dough depending on time and temperature – starch gelatization, protein denaturation, enzyme inactivation, gas expansion, evaporation of moisture from the crumb and crust. Varying the baking time and temperature the bread physical-chemical parameters and volatile compounds profile change significantly. The research was accomplished on triticale flour blend, which was made from whole grain triticale, rye, hull-less barley flour and rice, maize flour. The aim of the research was to investigate the influence of baking temperature on the physical-chemical properties and volatile compounds profile of triticale bread. Triticale bread was baked 45 minutes at 160±10 °C, 200±10 °C and 240±10 °C temperature. Hardness, stickiness, colour (L* a* b*), moisture, water activity, pH and titrable acidity of triticale bread crumb using standard methods were evaluated, but only colour (L* a* b*) of bread crust was evaluated during research. The volatile compounds profile was identified in bread crumb and crust separately. During research it was established that increasing baking temperature the bread crumb moisture, water activity, stickiness and pH decreased, but hardness and acidity increased. Variation of the baking temperature has significant influence (p=0.02) on bread crumb and crust colour. In the bread crumb and crust totally 26 volatile compounds were detected. There were identified 13 volatile compounds – common in all bread crumb and crust samples. All 26 volatile compounds belong to alcohols, aldehydes, acids, terpenes and ketones. Therefore it was ascertained that the baking temperature has significant influence (p<0.05) on the quality of triticale bread.

Keywords: triticale bread, quality, volatile compounds.

Introduction

Triticale (Triticosecale wittmack) is the first man-made cereal produced by crossing wheat (Triticum spp.) and rye (Secale cereal L.). The nutrition value of triticale is close to that of wheat and rye (Salmon et al., 2002). In order to extend the product assortment and improve their nutrition value, there can be used triticale, hull-less barley, buckwheat, hull-less oat and other grain flour that are used elsewhere in the world (Taketa et al., 2004). For expanding the range of bakery production in the world there are being developed various recipes for product enriching with fibre, proteins, vitamins and other nutrients for a healthier diet. It can be done making a flour blend from whole grain triticale, rye, hull-less barley, rice and maize flour (Sabovics, Straumite, 2012).

In bread making, baking is a key step in which the raw dough piece is transformed into a light, porous, readily digestible and flavourful product, under the influence of heat. During baking, the most apparent interactions are volume expansion, crust formation, inactivation of yeast and enzymatic activities, protein coagulation and partial gelatinisation of starch in dough (Therdthai et al., 2002). The quality of baked product is affected by the time and temperature of the baking process (Mondal, Datta, 2008).

The baking temperature can affect the bread texture and surface colour. The formation of colour in bakery products during baking is widely known as browning. Browning is the result of non-enzymatic chemical reactions which produce coloured compounds during the baking process: such reactions are the Maillard reaction and caramelization (Purlis, 2010).

Taste, smell and the flavour are undoubtedly the most important attributes determining the quality of bread or baked cereals in general and one of the most important attributes influencing the acceptance of the consumer (Hansen, Schieberle, 2005). Flavour is usually the result of the presence, within complex matrices, of many volatile and non-volatile components possessing diverse physical-chemical properties. Whereas, the non-volatile compounds contribute mainly to the taste, the volatile ones influence both – taste and aroma. A vast array of compounds may be responsible for the aroma of the food products, such as alcohols, aldehydes, esters, dicarboxyls, short to medium-chain free fatty acids, methyl ketones, lactones, phenolic compounds and sulphur compounds (Urbach, 1997).

For volatile compound investigation in bread samples can be used a solid-phase microextraction (SPME). Solid-phase microextraction is a relatively new sample preparation technique (Pawliszyn, 1997) before analysis through gas chromatography (Hook et al., 2002). SPME shows some advantages over other widely used techniques: solvent-free and rapid sampling, low cost, and higher sensitivity (Pawliszyn, 1997).

The aim of the research was to investigate the influence of baking temperature on the physical-chemical properties and volatile compounds profile of triticale bread.

Materials and Methods

Experiments were carried out at the Department of Food Technology in the Latvia University of Agriculture.

Triticale, rye and hull-less barley crops cultivated at the Priekuli Plant Breeding Institute (Latvia), rice and maize flour purchased from Joint Stock Company Ustuniu Malunas (Lithuania) were used in the current study.
Triticale, rye and hull-less barley were ground in a laboratory mill Hawos (Hawos Kornmühlen GmbH, Germany) obtaining whole grain fine flour.

Flour blend was made from 60% of whole grain triticale, 15% of whole grain rye, 15% of whole grain hull-less barley, 5% of rice and 5% of maize flour. Ingredients, such as sugar, salt, water and yeast were included in dough formulation in order to improve sensory properties and keep quality of bread.

In the research two dough samples using sugar, salt, dried yeast and water, mixed for 8 and 10 min (dough temperature 25 °C) were prepared. Dough samples were fermented 30 min at temperature 35 °C. For baking process three temperatures – 160±10, 200±10 and 240±10 °C were chosen using one baking time 45 min. The following physical parameters were analysed in obtained triticale bread after cooling it to room temperature 21±2 °C.

- **Moisture content** of bread crumb was determined using air-oven method (AACC method 44-15.02).
- **pH** of bread crumb was measured by JENWAY 3510 pH-meter (Bibby Scientific Ltd., UK).
- **Titratable acidity** of bread crumb was determined by titration where the sample (5 g) was dissolved and dispersed in 50 mL distilled water. Phenolphthalein indicator 0.5 mL 1% was added and titrated with standardized 0.1N NaOH until a faint pink colour endures for 30 seconds.
- **Hardness and stickiness** (N) of bread crumb samples were determined on the Texture Analyser – TA.XT.plus Texture Analyser (Stable Micro Systems Ltd., UK), using a modified version of AACC method 74-09.01.
- **Water activity** of bread crumb was determined by standard ISO 21807:2004, AquaLab LITE device (Decagon Devices, Inc., USA).
- **Colour** of bread crumb and crust samples were measured in CIE L* a* b* colour system using Tristimulus Colorimeter (ColorTec Associates, Inc., USA). Colour Tec PCM/PSM colour values were recorded as L* (brightness) – the vertical co-ordinate runs from L* = 0 (black) through grey to L* = 100 (white); a* (-a, greenness, +a, redness) – the horizontal co-ordinate, that runs from -a* (green) through grey to +a* (red) and b* (-b, blueeness, +b, yellowness) – another horizontal co-ordinate, that runs from -b* (blue) through grey to +b* (yellow) (Coultate, 2009). The measurements were repeated on different randomly selected locations at the surface of each crumb and crust sample (n=10).

**Volatile compounds** in bread crumb and crust were extracted using solid-phase microextraction (SPME) in the combination with gas chromatograph/mass spectrometry. Fiber was coated with a thin polymer film – Carboxen/Polydimethylsiloxane (CAR/PDMS). The film thickness is 85 μm with bipolar polarity (Supelco, Inc., USA). Five grams of sample were placed in 20 mL vial (small glass bottle). Extraction time is 65 min at 40 °C (incl., pre-incubation without the fibre for 15 min, 40 °C). Volatile compounds from fibre were thermally desorbed in the injector of gas chromatograph PerkinElmer 500 GC/MS with a capillary column Elite-Wax ETR (60 m×0.25 mm i.d.; DF 0.25 μm). The details of the program used in GC/MS analysis are following: the initial temperature was 40 °C, held for 7 min, then ramped from 40 °C to 160 °C at a rate of 6 °C/min, and from 160 °C to 210 °C at a rate of 10 °C/min with the holding time for 15 min. Mass spectrometer in Electron impact ionization mode was set on 70 eV as the electron energies, while the ion source temperature was set to 250 °C and inlet line temperature was set to 250 °C. Helium (he) was used as carrier gas at a constant flow of 1 mL min⁻¹. Acquisition parameters in full scan mode: scanned m/z 40–300. Compounds were identified by comparing their mass spectra with mass spectral library Nist98.

The results (mean, standard deviation, p value) were processed by mathematical and statistical methods. Data were subjected to one-way analysis of variance (ANOVA) by Microsoft Office Excel 2007; significance was defined at p<0.05. Principal component analyses were processed by MultiBase2014.

**Results and Discussion**

The comparison of baking temperature effect on changes of physical-chemical parameters and volatile compounds between the samples was done in the research. The triticale bread moisture and water activity are summarised in Table 1. In the baking time occurs evaporation of moisture and as well as decrease of the water activity.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Moisture, %</th>
<th>Water activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>M8-F¹</td>
<td>47.08±0.07</td>
<td>0.961±0.002</td>
</tr>
<tr>
<td>M8-C45-T160³</td>
<td>44.76±0.09</td>
<td>0.960±0.002</td>
</tr>
<tr>
<td>M8-C45-T200³</td>
<td>43.74±0.07</td>
<td>0.959±0.001</td>
</tr>
<tr>
<td>M8-C45-T240³</td>
<td>42.04±0.10</td>
<td>0.954±0.001</td>
</tr>
<tr>
<td>M10-F²</td>
<td>47.03±0.04</td>
<td>0.961±0.002</td>
</tr>
<tr>
<td>M10-C45-T160³</td>
<td>44.57±0.09</td>
<td>0.960±0.001</td>
</tr>
<tr>
<td>M10-C45-T200³</td>
<td>43.72±0.08</td>
<td>0.960±0.002</td>
</tr>
<tr>
<td>M10-C45-T240³</td>
<td>42.12±0.03</td>
<td>0.953±0.001</td>
</tr>
</tbody>
</table>

¹ moisture of fermented dough before baking, dough mixed 8 min; ² moisture of fermented dough before baking, dough mixed 10 min; ³ M8 means dough mixed 8 min, C45 – baking time (45 min), T160 – baking temperature (160 °C).

Smaller moisture losses comparing to the fermented dough before baking were found in sample M8-C45-T160 (2.32%) and M10-C45-T160 (2.46%), but the highest losses of moisture were in samples M8-C45-T240 and M10-C45-T240, as a result the moisture of samples decreased by 5.04% and 4.91%,
respectively. After obtained data evaluating, it could be concluded that not significant moisture evaporation for 8 min mixed dough, baked for 45 min at 160 °C, was found comparing with the sample M10-C45-T160. If fermented semi-finished product was baked at temperature 240 °C, moisture evaporation was the most intensive in M8-C45-T240 sample comparing with M10-C45-T240 sample. The water activity decreases with the increase of baking temperature. The changes in water activity basically paralleled the changes in moisture content, which was in agreement with study of Mathlouthi (2001) and Jakubczyk et al. (2008). Increasing the baking temperature also increases the acidity of bread, but pH decreases (Table 2).

### Table 2

<table>
<thead>
<tr>
<th>Sample</th>
<th>Acidity, °</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>M8-F</td>
<td>3.27±0.06</td>
<td>5.22±0.006</td>
</tr>
<tr>
<td>M8-C45-T160</td>
<td>3.30±0.04</td>
<td>5.21±0.008</td>
</tr>
<tr>
<td>M8-C45-T200</td>
<td>3.32±0.04</td>
<td>5.19±0.007</td>
</tr>
<tr>
<td>M8-C45-T240</td>
<td>3.40±0.05</td>
<td>5.09±0.004</td>
</tr>
<tr>
<td>M10-F</td>
<td>3.30±0.04</td>
<td>5.18±0.006</td>
</tr>
<tr>
<td>M10-C45-T160</td>
<td>3.34±0.02</td>
<td>5.16±0.003</td>
</tr>
<tr>
<td>M10-C45-T200</td>
<td>3.40±0.01</td>
<td>5.09±0.004</td>
</tr>
<tr>
<td>M10-C45-T240</td>
<td>3.47±0.03</td>
<td>5.07±0.004</td>
</tr>
</tbody>
</table>

1 moisture of fermented dough before baking, dough mixed 8 min; 2 moisture of fermented dough before baking, dough mixed 10 min; 3 M8 means dough mixed 8 min, C45 – baking time (45 min), T160 – baking temperature (160 °C).

The highest increase of titratable acidity was found in M8-C45-T240 and M10-C45-T240 samples, comparing with acidity in the fermented dough. Evaluating the data of moisture, water activity, acidity and pH, it can be seen that the baking temperature has significant influence (p<0.05) on changes of bread moisture, water activity, acidity and pH parameters. After bread cooling the bread crumb hardness and stickiness were analysed (Table 3). As evidence by the experimental results, the baking temperature has significant effect (p<0.05) on the crumb hardness and stickiness.

The softest (7.35±0.95 N and 7.79±0.82 N) bread crumb was acquired for triticale bread baked for 45 min at the temperature 160 °C, but the hardest bread crumb – for bread baked for 45 min at the temperature 240 °C. Comparing M8-C45-T160 and M8-C45-T240 samples, it can be seen that the bread crumb was harder by 12.01 N. As a result the mixing time does not affect the bread crumb hardness, but bread hardness mainly depends on the baking temperature. Das et al. (2012) in their research found that by increasing the baking temperature increases the hardness of the bread, losing part of the moisture in baking process.

### Table 3

<table>
<thead>
<tr>
<th>Sample</th>
<th>Crumb hardness, N</th>
<th>Crumb stickiness, N</th>
</tr>
</thead>
<tbody>
<tr>
<td>M8-C45-T160</td>
<td>7.35±0.95</td>
<td>-0.82±0.06</td>
</tr>
<tr>
<td>M8-C45-T200</td>
<td>14.5±0.83</td>
<td>-0.56±0.04</td>
</tr>
<tr>
<td>M8-C45-T240</td>
<td>19.36±0.92</td>
<td>-0.38±0.03</td>
</tr>
<tr>
<td>M10-C45-T160</td>
<td>7.79±0.82</td>
<td>-0.79±0.06</td>
</tr>
<tr>
<td>M10-C45-T200</td>
<td>14.92±0.51</td>
<td>-0.51±0.03</td>
</tr>
<tr>
<td>M10-C45-T240</td>
<td>19.81±0.25</td>
<td>-0.31±0.04</td>
</tr>
</tbody>
</table>

1 Sample description: M8 means dough mixed (8 min), C45 – baking time (45 min), T160 – baking temperature (160 °C).

Evaluating triticale bread crumb stickiness after baking, it can be concluded that more sticky crumb was for M8-C45-T160 and M10-C45-T160 samples, presenting the negative force value -0.82±0.06 N and -0.79±0.06 N, respectively. Increasing the baking temperature the stickiness of the bread crumb decreases, where M8-C45-T240 (-0.38±0.03 N) and M10-C45-T240 (-0.31±0.04 N) showed lower stickiness of the bread crumb. The correlation analysis shows that the triticale flour blend crumb hardness (r=0.983) and stickiness (r=0.981) have strong negative correlation on the moisture content of the bread. Thus, it can be concluded that the moisture content in bread affects the crumb hardness and stickiness.

The triticale bread crumb and crust colour was measured after baking, taking into account the baking temperature. The baking temperature has significant influence (p<0.05) on the bread crumb and crust colour changes.

Triticale bread crumb L* colour component value (Fig. 1) at different baking temperatures of all baked samples was significantly different (p<0.05).

![Figure 1. The changes of bread crumb and crust L* colour component value in different baking temperatures](image-url)

<table>
<thead>
<tr>
<th>Sample</th>
<th>L* value</th>
</tr>
</thead>
<tbody>
<tr>
<td>M8-C45-T160</td>
<td>58</td>
</tr>
<tr>
<td>M8-C45-T200</td>
<td>55</td>
</tr>
<tr>
<td>M8-C45-T240</td>
<td>53</td>
</tr>
<tr>
<td>M10-C45-T160</td>
<td>56</td>
</tr>
<tr>
<td>M10-C45-T200</td>
<td>53</td>
</tr>
<tr>
<td>M10-C45-T240</td>
<td>55</td>
</tr>
</tbody>
</table>

The bread crumb becomes darker increasing the baking temperature. The dough mixing time did not have significant affect (p>0.05) on crumb colour component.
L* value. For example, the crumb L* colour component value of sample M8-C45-T160 is 57.40±0.66 and of sample M10-C45-T160 – 57.12±0.87 was detected. Triticale bread baked for 45 min at 240 °C, apart from the previous dough mixing time, the colour of crumb and crust was obtained comparing to the other samples. After 45 min of baking at 240 °C the crust colour component L* value was 1.8 times lower in comparison with the crust colour of M8-C45-T160 and M10-C45-T160 samples. The baking temperature has no significant impact (p=0.06) on bread crumb dark/light shades, but significantly impacts (p=0.02) crust colour of all samples. Differences among the bread crumb and crust colour was obtained during research, as a result the colour of crust becomes darker increasing the baking temperature. Such interconnection was observed, because at higher temperature caramelization and Maillard reaction occurs faster (Martins et al., 2000).

The value of colour compound a* of bread crumb was in range from 0.50 to 0.76 (Fig. 2), but of bread crust – from 2.91 to 5.41. Increasing the baking temperature, there are no significant (p=0.06) changes of crust colour component a* value, but the baking temperature significantly affects (p=0.03) the crust colour. The colour value of the crust colour component a* decreases by about 2.27±0.14 from sample M8-C45-T160 to M8-C45-T240.

![Figure 2. The changes of bread crumb and crust colour compound a* value during baking](image)

The triticale bread crumb colour component b* value was in the range from 24.92±0.66 (M10-C45-T240) to 21.12±0.75 (M8-C45-T160) (Fig. 3) and was not significantly affected (p=0.07) by dough mixing time and bread baking temperature. Baking temperature has significant influence on crust colour component b* value changes. The highest intensity of yellow colour was found for the crust samples M8-C45-T160 (21.12±0.75) and M10-C45-T160 (21.75±0.79), but the lowest intensity of yellow colour was found for the crust samples M8-C45-T240 and M10-C45-T240, as 8.14±0.80 and 7.94±0.31 respectively. Increasing the baking temperature, component b* value in crumb samples increases, but in crust – decreases. It means that the bread crumb at higher baking temperature becomes yellowish. The yellow colour in the crust decreases increasing the baking temperature and this is mainly due to the fact that the rate of brown pigment formation increases with temperature (Das et al., 2012).

![Figure 3. The changes of bread crumb and crust b* colour compound value during baking](image)

1 – M8-C45-T160, 2 – M8-C45-T200, 3 – M8-C45-T240, 4 – M10-C45-T160, 5 – M10-C45-T200, 6 – M10-C45-T240

The L* a* b* colour values change similarly in all samples, what can be explained by moisture loses and brown colour formation during baking.

The volatile profile of different bread types has been widely investigated during the past years. These studies demonstrate that bread flavour is composed by different volatile compounds, belonging to several chemical classes, mainly heterocyclic compounds, alcohols, aldehydes, ketones, etc. Depending on the characteristic of each kind of bread, volatile compounds are present in well-defined ratios (Rehman et al., 2006; Bianchi et al., 2008).

Analysing of the volatile compounds in bread crumb and crust after baking overall there were identified 26 volatile compounds. All bread samples, which were baked at three different temperatures, contain 13 volatile compounds in common, identified both in crumb and crust. Among identified volatile compounds seven were alcohols (cyclobutanol, 4-penten-2-ol, 1-hexanol, 3-methyl-1-butanol, 3-methyl-2-hexanol, phenylethyl alcohol and maltol), two aldehydes (hexanal and heptanal), two acids (acetic acid and hexanoic acid), one ketone (3-hydroxy-2-butanoate) and one terpene (carvone).

From all identified volatile compounds in bread crumb and crust samples the highest peak areas were for three alcohols – 4-penten-2-ol, 3-methyl-1-butanol and 1-hexanol, their peak areas are shown in Table 4. The peak areas of alcohols (4-penten-2-ol, 1-hexanol and 3-methyl-1-butanol) are higher in bread crumb than in crust. But there can be observed that, the peak areas of alcohols decrease in bread crumb and crust increasing baking temperature, which is related to volatility of alcohols. According to Schieberle (1996) the amount of flavour compounds formed in bread can be affected by yeast amount and activity, fermentation and baking time and temperature.
The volatility of alcohols is dependent on the temperature in which it starts disappearing (boiling point), such as 4-penten-2-ol volatility intensively occurs in 134–137 °C, 3-methyl-1-butanol – 131 °C, but 1-hexanol from sample starts disappear at 155–159 °C (Janežič et al., 2006). It also explains why peak areas in the bread crust of 4-penten-2-ol and 3-methyl-1-butanol are more loss than in crumb increasing the baking temperature. The highest peak areas in bread crumb and crust of 4-penten-2-ol are identified in samples M8-C45-T160 and M10-C45-T160, but smaller ones – M8-C45-T240 and M10-C45-T240. The same regularities are observed for 3-methyl-1-butanol and 1-hexanol. The peak areas of 4-penten-2-ol in the sample M8-C45-T160 of crumb and crust are 3.0 to 3.3 times higher than in the sample M8-C45-T240. The peak areas of 3-methyl-1-butanol in bread crumb are 2.1 to 4.4 times higher than in crust. Volatile compounds distribution in the triticale flour blend bread crumb and crust, depending on baking temperature is shown in Figure 4. Evaluating the principal component analysis of the data, the distribution of volatile compounds depending on baking temperature explained 61.4% and 19.2% of variation on PC1 and PC2, respectively. Variation of PC1 shows bread crumb and crust samples, while PC2 represents the volatile compounds dispersion in bread crumb and crust samples.

**Bread crumb**
1 – M8-C45-T160; 3 – M10-C45-T160; 5 – M8-C45-T200; 7 – M10-C45-T200; 9 – M8-C45-T240; 11 – M10-C45-T240

**Bread crust**
2 – M8-C45-T160; 4 – M10-C45-T160; 6 – M8-C45-T200; 8 – M10-C45-T200; 10 – M8-C45-T240; 12 – M10-C45-T240

*MC – crumb; **GC – crust

---

**Table 4**

<table>
<thead>
<tr>
<th>Samples</th>
<th>4-penten-2-ol (PAUx10^6)</th>
<th>3-methyl-1-butanol (PAUx10^6)</th>
<th>1-hexanol (PAUx10^6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M8-C45-T160</td>
<td>328.31±3.00</td>
<td>121.07±0.59</td>
<td>59.85±0.59</td>
</tr>
<tr>
<td>M8-C45-T160</td>
<td>187.95±3.04</td>
<td>61.77±2.29</td>
<td>22.07±0.77</td>
</tr>
<tr>
<td>M8-C45-T200</td>
<td>214.97±2.80</td>
<td>103.47±0.75</td>
<td>51.78±0.54</td>
</tr>
<tr>
<td>M8-C45-T200</td>
<td>171.68±2.37</td>
<td>43.72±1.17</td>
<td>17.76±0.70</td>
</tr>
<tr>
<td>M8-C45-T240</td>
<td>98.21±3.96</td>
<td>72.91±0.30</td>
<td>21.62±1.64</td>
</tr>
<tr>
<td>M8-C45-T240</td>
<td>51.96±2.11</td>
<td>16.47±2.90</td>
<td>4.13±0.85</td>
</tr>
<tr>
<td>M10-C45-T160</td>
<td>329.86±4.96</td>
<td>121.66±0.98</td>
<td>68.38±0.35</td>
</tr>
<tr>
<td>M10-C45-T160</td>
<td>190.83±2.20</td>
<td>83.13±0.07</td>
<td>32.13±0.88</td>
</tr>
<tr>
<td>M10-C45-T200</td>
<td>254.25±4.97</td>
<td>108.37±0.24</td>
<td>60.80±0.88</td>
</tr>
<tr>
<td>M10-C45-T200</td>
<td>175.67±2.50</td>
<td>47.17±0.06</td>
<td>20.17±0.32</td>
</tr>
<tr>
<td>M10-C45-T240</td>
<td>108.29±3.65</td>
<td>85.76±0.03</td>
<td>30.71±0.46</td>
</tr>
<tr>
<td>M10-C45-T240</td>
<td>56.89±2.24</td>
<td>24.55±0.12</td>
<td>10.46±0.51</td>
</tr>
</tbody>
</table>

---

**Figure 4. Distribution of volatile compounds in the crumb and crust of the triticale bread samples**
The baking time has significant impact on volatile compound formation, because F=26.50>F_{crit}=1.53 with a probability of p<0.05 (95%). In the bread crust samples (mixed 8 and 10 min and baked 45 min at 240 °C) were detected the most pronounced volatile odour. In this samples more volatile compounds with higher peak areas are such as methylpirazine, 2-pentylfuran, hexanoic acid, maltol, furfural, 5-methyl-2-furfural, 2-furanmethanol, acetic acid, 3-hydroxy-2-butanone, and heptanal. Some of these volatile compounds are forming in fermentation time, but most of them in caramelization and Maillard reaction. Volatile compounds detected in triticale bread crust forms bread aroma profile, which includes fruit (4-penten-2-ol), malty (3-methyl-1-butanol and 2-methyl-1-propanol), honey (phenylethyl alcohol), green (hexanal, 1-hexanol), caramel (maltol), sweet (hexanoic acid, 3-methyl butanoic acid) aroma. But volatile compounds identified in bread crust supplement aroma profile with green bean (2-pentylfuran), bread (furfural) and butter (2-pentylfuran, 3-hydroxy-2-butanone) notes.

Conclusions
Baking temperature has significant influence (p<0.05) on changes of bread moisture, water activity, acidity and pH parameters, as well as hardness of bread crumb. Baking temperature significantly influences triticale bread crust colour and volatile compounds formation in the crumb and crust. Totally in all samples baked at different temperature were identified 26 volatile compounds. Detected volatile compounds in bread crumb form bread aroma profile, which includes fruit, malty, honey, green, caramel, sweet aroma, but volatile compounds identified in bread crust supplement aroma profile with green bean, butter and bread notes.

Acknowledgment
This research has been prepared within the frameworks of the ESF project „Formation of the Research Group in Food Science“, No. 2009/023/1DP/1.1.1.2.0/09/APIA/ V1AA/122. ESF grant „The support for implementation of LLU doctoral studie“, No. 2009/0180/1DP/1.1.2.1.2/09/APIA/ V1AA/017 and State Research Programme NatRes Project No. 3, „Sustainable use of local agricultural resources for development of high nutritive value food products (Food)“.

References
SENSORY, COLOUR AND STRUCTURAL PROPERTIES OF PANCAKES PREPARED WITH PEA AND BUCKWHEAT FLOURS

Ilze Beitane¹, Gita Krumina-Zenture¹, Irisa Murniece²

¹ Department of Nutrition, Faculty of Food Technology, Latvia University of Agriculture, 2 Liela Street, Jelgava, Latvia,
² Department of Food Technology, Faculty of Food Technology, Latvia University of Agriculture, 2 Liela Street, Jelgava, Latvia

Abstract

Pea and buckwheat flours, that are gluten free, possess nutritionally important amino acids, therefore they are useful for people suffering from celiac disease. The treatment of celiac disease is gluten free diet, thus it is important to produce gluten free products with high nutritional value. The aim of the research was to evaluate the effect of pea and buckwheat flour on colour, sensory and structural properties in pancakes. The results showed that during and after frying process pancakes kept their shape when 100% of pea flour was used. The structure of pancakes was changed from harder to the softer and was floury due to the pea flour replacement to the buckwheat flour. When the pancake was prepared with 100% of buckwheat flour, the pancakes fell apart – did not keep the shape properly during frying. The sensory analysis results showed that pea and buckwheat flour affected pancake colour and taste. When higher amount of buckwheat flour was used in pancake preparation, the colour of pancakes changed to darker, the structure was softer and buckwheat flavour was predominant. The experienced panelists indicated that pancakes with 40% and 60% supplemented buckwheat flour were the most pleasant during sensory analysis. Similar tendency was obtained with results of colour (CIE Lab system) and texture analysis. L* and b* colour indices of pancakes had decreasing tendency when the amount of buckwheat flour was increased.

Keywords: pea flour, buckwheat flour, sensory properties, texture, colour.

Introduction

Celiac disease is one of the most common lifelong disorders, affecting 1% of the population worldwide (Catassi, Yachha, 2009). It is a digestive tract disease that damages the small intestine and interferes with the absorption of nutrients from food (De la Hera et al., 2013). Gluten is a major protein component of wheat flour which is responsible from dough forming characteristics in bakery industry (Torbica et al., 2010), affects elastic properties of dough and contributes to the overall appearance and crumb structure of many baked products (Torbica et al., 2012). However, gluten must be eliminated from the diet of patients with celiac disease. Therefore it is important to produce gluten free products with high nutritional value. Many researchers have studied the substitution of gluten by ingredients with the similar functional properties (Sciarini et al., 2008). Most of the gluten free products are on starch or rice basis with addition of different types of hydrocolloids (Torbica, 2010).

Legumes have an important place from the nutritive point of view (Kohajdová et al., 2013), they are as good sources of proteins, carbohydrates, several water soluble vitamins (Sreerama et al., 2012) and minerals such as calcium, iron, zinc and magnesium (Kaur et al., 2007; Amarakoon et al., 2012). Legumes have been identified as low glycaemic index foods (Bornet et al., 1997), which proteins can be successfully used in baked products to obtain a protein-enriched product with improved amino acids balance (Mohammed et al., 2012). Legume flours were successfully applied as functional ingredients in cereal based foods such as bread (Hefwawy et al., 2012), cake (Gómez et al., 2008), cracker biscuits (Kohajdová et al., 2013) and spaghetti (Arab et al., 2010).

Field pea is one of the oldest domesticated food crops, which is grown widely as a cool season grain legume (Wang et al., 2011) and which is an excellent source of complex carbohydrates, protein, dietary fibre, vitamins and mineral micronutrients (Wang et al., 2010; Amarakoon et al., 2012). The starch and protein content of peas range between 30–50% and 20–25% of the dry matter, respectively (Simsek et al., 2009; Pieczyk et al., 2012). Kohajdová et al. (2013) indicated that inclusion of pea flour to wheat dough resulted in increased water absorption capacity and dough development time, in addition incorporation of higher levels of pea flour (20% and 30%) into crackers significantly reduced volume index, width to spread ratio of final products.

Buckwheat is recognised as an important functional food in some countries such as China, Japan and Taiwan and can be used to replace rice or potatoes in the regular meal (Lin et al., 2009). Buckwheat proteins consisted mainly of globulins (up to 50%) and albumins (about 25%) (Choi et al., 2006), they are known for their well-balanced amino acid content (Ikeda, Asami, 2000) and are particularly rich with essential amino acids as lysine and arginine (Choy et al., 2013) as well as they are gluten free which makes them useful for patients suffering from celiac disease (Ikeda, 2002). In addition, buckwheat was found to be a prebiotic, because the research of Prestamo et al. (2003) showed that buckwheat could increase lactic acid bacteria in rat intestine. In general buckwheat, which is added to food as a supplement, can provide beneficial health effects and prevent food from oxidation during processing (Lin et al., 2009).

Pea and buckwheat flours, that are gluten free, possess nutritionally important amino acids, therefore they are useful for people suffering from celiac disease. Choy et al. (2013) incorporated buckwheat flour into noodle formulation instead of wheat flour in order to analyse
the colour of final products. They found that the brightness of noodles was low that can be regard to brownish colour. Lin et al. (2009) indicated that buckwheat enhanced wheat bread exhibited lower brightness and higher redness and yellowness. The above mentioned research findings showed that pea and buckwheat possess unique nutritional properties and in the development of new products, the addition of pea and buckwheat flour would enrich nutritional composition of the product and thus would be beneficial for our health. However, replacement of wheat flour with pea and buckwheat flour would lead to some changes on rheological and sensory properties. Therefore, the aim of the research was to evaluate the effect of pea and buckwheat flour on colour, sensory and structural properties of pancakes.

Materials and Methods

Materials

Pea flour (PF) and buckwheat flour (BF) were purchased from Fasma, Lithuania. Sugar, baking powder, milk with fat content 2.5% and refined rapeseed oil for dough preparation of pancakes were obtained from the local food market.

The procedure of pancake preparation

At the beginning of pancake preparation, 100% of pea flour (PF) was used and then pea flour was replaced by buckwheat flour (BF) in following PF : BF ratios: 80 : 20, 60 : 40, 40 : 60 and 20 : 80. Because of radical changes in structural properties of the final product, pancakes prepared with 100% of buckwheat flour were excluded in this research.

In the preparation of the dough, all ingredients were mixed to reach smooth dough consistency; then dough was kept for 20 min (resting time) then fried for 8±1 min on frying pan where oil temperature was adjusted to 150±2 °C. Pancakes were served at 65±2 °C to determine sensory properties. According to the added amount of pea and buckwheat flour, the following abbreviations for the samples in current research were used:

- 100% PF – pancakes with 100% of pea flour;
- 80% PF+20% BF – pancakes with 80% of pea and 20% of buckwheat flour;
- 60% PF+40% BF – pancakes with 60% of pea and 40% of buckwheat flour;
- 40% PF+60% BF – pancake with 40% of pea and 60% of buckwheat flour;
- 20% PF+80% BF – pancake with 20% of pea and 80% of buckwheat flour.

Sensory evaluation of pancakes

In order to evaluate differences among pancakes prepared with different amount of buckwheat flour, sensory assessment was performed. Eight experienced staff members from Latvia University of Agriculture Faculty of Food Technology (females between 33–62 years old) were selected. They were selected according to their willingness, availability, motivation and previously demonstrated capability to work as a member of a sensory panel.

Five sensory properties – aroma, taste, colour, hardness and structure were analyzed using a line scale (ISO 4121:2003). Pea and buckwheat aroma, pea and buckwheat taste, yellow and grey colour was followed from ‘not perceptible’ to ‘strongly perceptible’. Hardness was defined from ‘soft’ to ‘strong’ and structure expressed from ‘porous’ to ‘dense’.

In the second part of sensory analysis, the ranking test (ISO 8587:2006) was used with the aim of ranking pancake samples in the following order: the most pleasant – the worst.

Colour measurement

Colour measurements of pancakes were carried out in quintuple using CIE Lab system. The obtained results were expressed in terms of CIE L*, a* and b* values. L* indicates brightness, a* represents red to green coordinates and b* represents blue to yellow coordinates of a product.

Structure analysis

Texture analyzer “TA.XT.plus Texture Analyser” (Stable Micro Systems Ltd., Surrey, UK) with a Warner–Bratzler shear blade with guillotine probe was used. The cut was performed perpendicularly to the main axis of the pancake until completely breaking. The peak force obtained was taken to be the result from the test (N). The cutting parameters: pre-test speed 1 mm s⁻¹; test speed 3 mm s⁻¹; post test speed 10 mm s⁻¹; cutting distance of 25 mm into the pancake sample. The maximum force required for sample cutting was calculated as an average of six measurements. Obtained data were processed in the Texture Exponent 32 programme.

Statistical analysis

The results were analyzed using one way and two way analysis of variance (ANOVA). T-test was applied to compare the mean values, and p-value at 0.05 was used to determine the significant differences. The correlation analysis was performed in order to determine the relationship between results of sensory analysis and measurements of colour and structure.

Results and Discussion

It is known that consumer behaviour about food choice is determined not only by nutritional value and healthy properties but also sensory evaluation of particular food product. Good quality pancake should possess pleasant pea or buckwheat taste and aroma as well as soft and porous structure. The changes of sensory properties of pancakes with pea flour to buckwheat flour ratios were shown in Figure 1.

The changes of sensory properties of pancakes were affected by the amount of buckwheat flour replaced with the pea flour. By increasing the amount of buckwheat flour the structure of pancakes became softer and denser, colour became darker and buckwheat taste as well as aroma – predominant.
Figure 1. Intensity of sensory properties of pancakes prepared with different ratios of pea flour to buckwheat flour

Evaluation of sensory properties of pancakes with pea and buckwheat flour showed that there were significant differences in intensity of pea and buckwheat taste and aroma, in addition to colour and hardness (p<0.05), but there was no significant difference in their structures (p>0.05). Pancake samples prepared with 100% PF had significant difference in terms of intensity of pea taste and aroma (p<0.05) when compared to other pancakes. The intensities of buckwheat taste and aroma, colour and hardness exhibited significant differences (p<0.05) when the pancakes produced with the highest amount of buckwheat flour (20% PF+80% BF). The structure of the same sample was softer and colour much darker when compared to the lightest grey colour obtained with the sample of 80% PF+20% BF. Focusing on the results of structure, pancake sample having 40% PF+60% BF ratio had significant difference (p<0.05) when compared to other samples in term of its soft consistence.

Sensory analysis showed that buckwheat flour addition into the pancake formula caused decreasing of pea taste and aroma, in other words, predominance of pea taste and aroma felt by experts was not so acceptable. Similar conclusions were reported by Kohajdová et al. (2013) in which higher levels of pea flour caused significant reduction in taste, odour and overall acceptance of final crackers due to higher intensity of leguminous taste and odour.

The sensory analysis of ranking showed that the most pleasant pancake samples were obtained with 40% PF+60% BF and 60% PF+40% BF and they were significantly different from other pancake samples (p<0.05).

In evaluation of the colour changes in pancake samples with different buckwheat flour supplementation ratio, sensory experienced staff determined similar results. When the buckwheat flour addition was increased in pancake preparation by replacing pea flour, the brightness (L*) of pancake decreased apparently (Fig.2). Similar results were obtained in the study of Lin et al. (2009) by substituting 15% of wheat flour with buckwheat flour in wheat bread preparation. It was the fact that buckwheat flour exhibited beige colour. Choy et al. (2013) and Chillo et al. (2008) reported that the L* values of instant noodles and spaghetti containing buckwheat flour significantly decreased when compared with control samples.

Decreasing of lightness in 20% PF+80% BF pancake preparation was more than 25% when compared with 100% PF pancake formulation. Pancake samples became much darker when buckwheat flour incorporation increased. On the other hand, Lin et al. (2009) reported that the dark colour of buckwheat incorporated wheat bread might be noticeable to attract consumer’s attention.

The correlation was found as very close (r=0.995) between the lightness measurements and experts assessments related to colour intensity in pancake samples. Buckwheat flour incorporation in pancake preparation resulted an increase in a* values from -3.58 to 2.27 (Fig. 3).

Similar findings were reported by Choy et al. (2013) where a* value changed from negative to positive value in instant noodles by increasing the buckwheat...
amount. Lin et al. (2009) determined similar results like an increase of redness value in wheat bread with the addition of buckwheat flour. In addition, differences were determined between unhusked and husked buckwheat supplemented wheat bread. The correlation analysis showed that there was close correlation ($r=0.998$) between measurements of $a^*$ value and results of sensory analysis. In evaluation of the $b^*$ value intensity in pancake samples, there was concluded that buckwheat flour significantly affected $b^*$ value by decreasing yellowness value in pancakes (Fig. 4). Similar conclusions were indicated by Torbica et al. (2012). In their research related to cookie production with rice and buckwheat flour, yellowness values slightly decreased by increasing the amount of buckwheat flour in cookie formulation.

Figure 4. The $b^*$ value intensity of pancakes prepared with different ratios of pea flour to buckwheat flour

When the 100% PF pancake sample was compared with the 20% PF+80% BF pancake sample, an increased amount of buckwheat flour caused more than 50% decrease on yellowness. Furthermore, close correlation ($r=0.974$) was determined between measurement of $b^*$ value and expert’s assessment of colour intensity for pancakes prepared with pea and buckwheat flour.

The results of colour measurements showed that the increased amount of buckwheat incorporation in pancake formulation significantly ($p<0.05$) affected $L^*$, $a^*$ and $b^*$ values. The hardness results of pancakes prepared with different ratios of pea flour to buckwheat flour were given in Figure 5. The adding of buckwheat in concentrations of 60% and 80% significantly decreased hardness values of pancake samples when compared to other samples. It was observed that an increase of buckwheat flour incorporation caused a decrease on hardness values of pancake samples. Choy et al. (2013) indicated that incorporating buckwheat flour significantly affected hardness of instant noodles which became softer.

Figure 5. Hardness of pancakes prepared with different ratios of pea flour to buckwheat flour

Whereas Kahajdová et al. (2013) established that addition of pea flour concluded in increased hardness of crackers. Therefore, it could be concluded that addition of buckwheat flour provided softer structure of pancakes. These changes were observed by experts too. The results of sensory analysis and structure analysis exhibited close correlation ($r=0.951$).

Conclusions

The sensory properties were affected by the amount of added buckwheat flour in pea flour pancakes. There were significant differences in intensity of pea and buckwheat taste and aroma, colour and hardness ($p<0.05$), but there was no significant difference in structure ($p>0.05$). Sensory analysis showed that buckwheat flour incorporation into the pancake formulation caused decreasing of pea taste and aroma – predominance of pea taste and aroma was not acceptable according to the evaluation of sensory panelists. The amount of added buckwheat in pancake formula significantly ($p<0.05$) affected $L^*$, $a^*$ and $b^*$ values as well as resulted softer structure on pancakes.

References


GLUTEN-FREE SOURDOUGH BREAD PREPARED WITH CHESTNUT AND RICE FLOUR

Ilkem Demirkesen Mert¹, Osvaldo H. Campanella²,³, Gulum Sunnun¹, Serpil Sahin¹

¹ Department of Food Engineering, Engineering Faculty, Middle East Technical University, Universi ted Mah, Ankara, Turkey, e-mail: gulum@metu.edu.tr
² Department of Agricultural and Biological Engineering, Engineering Faculty, Purdue University, West Lafayette, IN, USA

Abstract
In order to meet the rising demand of celiac patients for high quality and healthier products, the old biotechnological process, sourdough technology, was applied in gluten-free bread-making. In present study, the effect of addition of different amounts of sourdough (0%, 20% and 40%, % flour basis) on the rheological behaviour of the gluten-free chestnut-rice dough formulations prepared with chestnut and rice flours as well as on the quality parameters (pH, total titratable acidity (TTA), firmness and volume) of chestnut-rice breads was pointed out. The increasing levels of sourdough addition reduced pH and increased TTA values of samples. The addition of sourdough decreased complex modulus (G*) of dough samples showing softening of dough. The highest specific volume and the lowest firmness values were obtained from gluten-free breads with the addition of 20% sourdough. However, higher level (40%) of sourdough addition had detrimental effect on volume and firmness of breads. The results of present study showed that addition of 20% of sourdough can be used to improve the quality of gluten-free chestnut-rice breads.

Keywords: chestnut flour, gluten-free bread, rice flour, sourdough.

Introduction
Celiac disease, which is an autoimmune disorder, occurs in genetically vulnerable individuals by exposure to cereal gluten proteins. Since gluten creates a continuous protein network which helps to retain gas produced from yeast fermentation and oven rise, lots of the gluten-free baked products on the market are of poor quality with low volume, and poor texture. For these reasons, gluten replacement is still remaining to be one of the most trivial tasks for cereal technologist and scientists (Moore et al., 2008; Demirkesen et al., 2010). Sourdough, a mixture of flour and water fermented with lactic acid bacteria and/or yeast, are used to improve the quality of wheat breads (Moroni et al., 2009). In order to meet the rising demand of celiac patients for high quality and healthier products, this old biotechnological process has lately been applied as a new approach in gluten-free bread-making. Chestnut flour contains high quality proteins with essential amino acids (4–7%), relatively high amount of sugar (20–32%), starch (50–60%), dietary fibre (4–10%), and low amount of fat (2–4%) (Demirkesen et al., 2010). It also includes some important vitamins such as vitamins E, C, B group and minerals such as potassium, phosphorous, magnesium, calcium, copper, iron, manganese and sulphur (Chenlo et al., 2007; Demirkesen et al., 2010). In addition, it has some important phenolics (gallic and ellagic acid) that have various positive health effects (Blaiotta et al., 2012). It has been stated that gluten-free breads prepared using chestnut-rice flour mixtures had higher volume and softer texture compared with those prepared with only chestnut or rice flour (Demirkesen et al., 2010; 2011; 2013a). In a recent study, it has also been observed that the staling of gluten-free rice breads was delayed by the partial replacement of rice flour with chestnut flour (Demirkesen et al., 2013b). The main objective of the present study was to investigate the effect of the addition of different amounts of sourdough (0%, 20% and 40%, % flour basis) on the rheological behaviour of gluten-free chestnut-rice dough formulations. In addition, the quality parameters (pH, total titratable acidity (TTA), firmness and volume) of chestnut-rice breads were determined.

Materials and Methods
Characterisation of ingredients

Chestnut flour was supplied by Kafkas Pasta Sekerleme San. & Tic. A.S. (Karacabey, Bursa, Turkey). Bob’s Red Mill Organic Brown Rice Flour (Milwaukie, OR, USA) was obtained from a local market. Sugar (sucrose), salt, vegetable oil (MarketPantry® vegetable oil, MN, USA) and instant yeast (RedStarYeast & Products, Milwaukee, WI, USA) were purchased from local markets. A gluten-free commercial starter named brown rice sourdough starter (L. plantarum) specifically designed for use in gluten-free sourdough breads was used. Emulsifier DATEM (diacetyltartaric acid esters of monoglycerides) (E) was obtained from Danisco Co., (Copenhagen, Denmark). Xanthan (X) and guar (G) were obtained from Sigma–Aldrich (St. Louis, MO, USA).

Sourdough (SD) preparation

The starter stain (0.6 g) was added to water (100 g), rice flour (70 g) and chestnut flour (30 g) mixture thereby giving a dough yield of 200 (Table 1). The resultant dough was mixed thoroughly by hand for 2 min, poured into large beakers, covered and then incubated in proofer (InterMetro Industries Co., Wilkes-Barre, PA) at 30 °C and 85% relative humidity (RH) for 72 hours. Then, different amount of the sourdough (20% and 40% flour basis) was added to bread formulations.

Bread making procedure

Gluten-free bread formulations used in this study are presented in Table 1. Percentages of ingredients were based on 100 g of flour amount.
During dough preparation, dry ingredients (chestnut flour, rice flour, instant yeast, sugar, salt and emulsifier) were mixed thoroughly, and then the melted shortening was added. Finally gum suspension and water were added slowly and mixed for 3 min at 85 rpm and then 2 min at 140 rpm using a mixer (Kitchen Aid, 5K45SS, Elk Grove Village, USA). After complete mixing, fermentation was done at 35 °C and 85% RH in a proofer (InterMetro Industries Co., Wilkes-Barre, USA) for 40 min. Following fermentation, gluten-free bread samples were baked in a rotatory electric oven (National Mfg. Co., Lincoln, USA) and breads samples (200 g each) were baked at 200 °C for 30 min. Gluten-free breads prepared with 30% of chestnut and 70% rice flour and without addition of sourdough were used as controls.

Rheological measurements

Dough samples for the rheological tests were prepared without adding any yeast to the formulation to avoid interference of bubble formation. The rheological measurements were conducted using a rheometer (ARG-2 Model, from TA Instruments, Newcastle, DE, USA) with a parallel plate geometry (40 mm diameter and 2 mm gap) at 25 °C. For the relaxation of the residual stresses, the dough was rested at room temperature for 20 minutes before testing. Frequency sweep experiments were carried out at 0.5% strain rate between 0.1 to 10 Hz. Results were expressed in terms of complex modulus (G*) values. All the rheological experiments were performed at least twice and their averages were reported in the study.

pH and TTA (total titratable acidity) in gluten-free sourdoughs and breads

The total titratable acidity (TTA) and pH values were determined in bread dough and breads. The pH values were measured from an aliquot of 10 g of sourdough, bread dough and bread crumb suspended with 100 mL distilled water. For the determination of TTA, these suspensions were titrated to against 0.1 N NaOH to a final pH value of 8.5. TTA was expressed as the amount of 0.1 N NaOH 10 g⁻¹ consumed in mL. Each analysis was done in triplicate and their averages were reported in the study.

Firmness

Baked loaves were allowed to cool for 1 h before obtaining measurements using the TA-XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY, USA). Samples in cubic shapes having dimensions of 25×25×25 mm were taken from the centre of bread and were compressed to 25% of its thickness with a cylindrical probe of diameter 25.4 mm (approved method 74–09, AACC 2000). The firmness, F (the force required to compress the sample) of the crumb was calculated from a force–distance graph. The measurements were done in duplicate.

Specific bulk volume

To determine specific volume, weight of loaves (g) was measured and bread volume (cm³) was determined by the rapeseed displacement method 20 min after cooling at 25 °C. Then, specific volume was calculated as the volume/mass ratio (cm³ g⁻¹) of bread according to (approved method 10–05, AACC 2000). The measurements were done in duplicate.

Statistical analysis

One-way ANOVA was used to determine whether gluten-free formulations affected firmness and specific volume of breads significantly (p≤0.05). If significant difference was found, means were compared by using the Tukey multiple comparison test (p≤0.05) by using Minitab (Version 16) software.

Results and Discussion

pH and TTA of sourdough, dough and bread

Data on acidification of gluten-free bread dough and breads prepared with/without the addition of different amounts of sourdough was compared in Table 2. In the lack of sourdough, control dough and bread samples had the highest pH and the lowest TTA values. The increasing levels of sourdough addition reduced pH and increased TTA values of samples. The similar findings have been reported by Crowley et al., 2002 and Moroni et al., 2011.

**Table 1**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Control (%)</th>
<th>20% SD</th>
<th>40% SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chestnut flour</td>
<td>30</td>
<td>24</td>
<td>18</td>
</tr>
<tr>
<td>Rice flour</td>
<td>70</td>
<td>56</td>
<td>42</td>
</tr>
<tr>
<td>SD</td>
<td>-</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>Water</td>
<td>163</td>
<td>143</td>
<td>123</td>
</tr>
<tr>
<td>Sugar</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Salt</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Oil</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Yeast</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>X-G gum blend</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

* SD – sourdough
** E – emulsifier DATEM

**Table 2**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dough pH</th>
<th>TTA</th>
<th>Bread pH</th>
<th>TTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.41±0.08</td>
<td>2.82±0.02</td>
<td>6.44±0.02</td>
<td>2.97±0.04</td>
</tr>
<tr>
<td>20% SD</td>
<td>5.32±0.21</td>
<td>5.41±0.01</td>
<td>5.42±0.06</td>
<td>5.23±0.07</td>
</tr>
<tr>
<td>40% SD</td>
<td>4.87±0.01</td>
<td>7.37±0.05</td>
<td>4.94±0.04</td>
<td>6.97±0.09</td>
</tr>
</tbody>
</table>

240
Rheological measurements
The complex modulus (G*) values of dough samples prepared with/without the addition of different amounts of sourdough can be seen in Figure 1.

Figure 1. Complex modulus (G*) of gluten-free dough samples prepared with/without the addition of different amounts of sourdough

The highest complex modulus over the whole frequency range was obtained from control chestnut-rice dough samples. The addition of sourdough decreased complex modulus (G*) of dough samples. Further decreases in the complex modulus (G*) were observed with the addition of higher amount of sourdough (40%). The decreases in complex modulus (G*) of dough samples showed that the addition of sourdough promoted softening of dough. Protein breakdown and the consequent release of small polypeptide during fermentation have also been identified as the major cause for the weakening of the protein-protein and protein-starch interactions resulting in impairing of elasticity of dough (Houben et al., 2010). Consequently, the decreases of complex modulus in gluten-free dough samples might be related to reduced water holding capacity of hydrolyzed proteins, which was induced by organic acids and enzymes released during the sourdough fermentation. Thus, the lack of sourdough fermentation, higher complex modulus (G*) values were obtained as compared to control dough samples. Drastic decreases in the degree of elasticity of dough by sourdough fermentation were also shown in different studies (Clarke et al., 2002; Houben et al., 2010; Galle et al., 2012).

Firmness and specific volume of breads
Firmness and specific volume of breads prepared with/without the addition of different amount of sourdough can be seen in Fig. 2–3.

The addition of sourdough improved the texture of gluten-free breads, beyond a certain amount. Therefore, the lowest firmness values were obtained from breads with the addition of 20% sourdough.

Figure 2. Firmness values of gluten-free dough samples prepared with/without the addition of different amounts of sourdough

Control: control chestnut-rice bread, 20% SD: bread prepared with addition of 20% sourdough, 40% SD: bread prepared with addition of 40% sourdough.

Control – control chestnut-rice bread, 20% SD – bread prepared with addition of 20% sourdough, 40% SD – bread prepared with addition of 40% sourdough.

The volume of gluten-free breads prepared with by the addition of 20% sourdough had comparable specific volume with control breads. However, further addition of sourdough deteriorated the quality of gluten-free
breads. Softer dough formation with the addition of sourdough might have facilitated greater bubble expansion upon fermentation process. Thus, the enhancement of specific volume and texture of gluten-free breads by the addition of certain amount of sourdough (20%) might be related to the improvement of the capacity of dough to retain CO₂. On the other hand, addition of higher level of sourdough (40%) had detrimental effect on volume and texture of breads since viscosity and viscoelastic properties of dough were not sufficient enough to allow bubble capture. Several previous studies also reported that sourdough addition might improve specific volume and texture of breads as long as it is used at certain amount (Crowley et al., 2002; Novotni et al., 2012).

**Conclusion**

Gluten-free breads formulated with the addition of different amounts of sourdough (0%, 20% and 40%) were evaluated using rheological and baking measurements. Rheological measurements showed that the addition of sourdough led to decreases in complex modulus (G*) of dough samples promoting softening of dough. According to baking tests, the breads prepared with the addition of 20% sourdough had the best quality parameters. However, higher level of sourdough addition led to some deterioration in quality parameters. Therefore, the results of the present study showed that the quality of gluten-free chestnut-rice breads can be successfully improved by the addition of 20% sourdough.

**References**

THE EFFECT OF SPRAY DRYING PROCESSING CONDITIONS ON PHYSICAL PROPERTIES OF SPRAY DRIED MALTODEXTRIN

Bau Koç¹, Figen Kaymak-Ertekî²

¹ Gaziantep University, NaciTopçuolu Vocational School, Gaziantep, TÜRKİYE, e-mail: kocbun@gmail.com
² Ege University, Faculty of Engineering, Food Engineering Department, 35100, Bornova, İzmir-TÜRKİYE

Abstract

Malto dex trins have wide applications particularly in the food industry. They have many functionalities including usage as wall material, dispersing aid, flavor carrier, bulking agent, viscosifier or fat replacer, and they exhibit only a slightly sweet taste. Malto dex trin was subjected to s pry drying t o determine the effect of spray drying conditions on moisture content, particle properties (particle size distribution and particle density) and bulk properties (bulk and tapped densities and porosity) of the powder product. Experiments have been performed in a lab scale spray dryer (BüchiLaborteknik AG, Flawil, Switzerland) using a full-factorial design to provide data and correlations that predict the powder properties as a function of the main operational variables of the spray dryer. The inlet (140 and 200 °C) and feed temperatures (10 and 50 °C), feed flow rate (2.1×10⁻⁴ and 9.6×10⁻⁴ kg s⁻¹) and atomizing air flow (1.3×10⁻⁴ and 1.9×10⁻⁴ m³ s⁻¹) were investigated as spray drying process variables. The effect of spray drying conditions on physical properties of powders was expressed with three dimensional response surface and perturbation graphs. Perturbation graphs revealed that atomizing air flow and inlet air temperature had more effect than feed temperature and feed flow rate on the physical properties of malto dex trin powder. The results showed that the Sauter mean diameter (D₃₂) was between 3.503 and 6.045 µm for malto dex trin powders.

Keywords: malto dex trin, spray drying, particle and bulk properties, particle size distribution.

Introduction

Spray drying is a well-established and widely used method for transforming a wide range of liquid food products into powder form. Spray-dried powders can be stored at ambient temperature for prolonged periods without compromising the powder stability. They are also cheaper to transport and easier to handle in manufacturing plants (Koç et al., 2010; Koç et al., 2011). However, caking or stickiness as one of the major degradation problems hindered the development of powders (Adhikari et al., 2007). The problem is mainly due to the existence of low molecular weight sugars with low glass transition temperatures. To produce food powders, using malto dex trins as a drying carrier is a popular method nowadays (Bhandari et al., 1997). Malto dex tin can significantly increase the glass transition temperature and reduce the hygroscopicity of dried products (Goula, Adamopoulos, 2008).

Malto dex trin, a common encapsulating material used in the food industry, is made by the hydrolysis of starch, and comes in the form of a white powder and has a sweet taste. Malto dex trins have particularly wide applications in the food industry especially in spray drying. They have many functionalities including usage as wall material, dispersing aid, flavor carrier and bulking agent. They are mainly used in materials that are difficult to dry -such as fruit juices, flavorings, and sweeteners- and to reduce stickiness, thereby improving the product stability (Bhandari et al., 1993; Bhandari et al., 1997; Roos, Karel, 1991). For this reason, spray drying conditions and physical properties of malto dex trin should be determined.

Physical properties of food powders including the particle shape, density and porosity, surface characteristics, diameter, and size (Kurozawa et al., 2009) can be affected by the spray drying temperatures and the type of atomizer, that are important in the storage, handling and final application of powder product (e.g. particle and bulk properties). Particle properties are directly related to physical properties of powder food products (Schubert, 1987). It is known that complex changes in the particles properties (size, shape, density and appearance) of droplets occur during spray drying and that the protection of these properties is related to the porosity and integrity of the microcapsules. With respect to morphology, the particles produced by spray drying generally show a smooth surface and are spherical in shape, have lowest surface-to-volume ratio (aroma retention), highest bulk densities (best packing) and best flowability (Kurozawa et al., 2009).

One of the most important physical parameters of powders with regard to handling is particle size. Particle size can influence flow out of storage bins, the blending of different components, compaction, and the segregation of a mixture, in which smaller particles stay distributed on the bottom and larger particles on top. In addition, these properties significantly influence the essential properties of food products such as smell, texture, and appearance. As particle size decreases, the increase in the particle surface area causes higher affinity with moisture and higher ability to agglomerate during the drying process. The knowledge of food density is of fundamental use for material properties studies and for industrial processes in adjusting storage, processing, packaging, and distribution conditions. Bulk density includes the volumes of the solid and liquid materials and all pores and is generally used to characterize a final product obtained by drying (Kurozawa et al., 2009).

In this study, it was aimed to determine the influence of spray drying process, in terms of inlet air and feed temperatures, feed flow rate and atomizing air flow on moisture content, water activity, particle properties (particle size distribution and particle density) and bulk properties (bulk and tapped densities, porosity,
flowability) of maltodextrin.

Materials and Methods

Maltodextrin (DE=8), used as the test material was supplied from Çağdaş Kimya, Turkey. Maltodextrin was dissolved in the distilled water and the solution containing 25% maltodextrin was used in the experiments.

Spray Drying

Experiments were conducted in a lab scale spray dryer (BüchiLabortechnik AG, Flawil, Switzerland). Maltodextrin solution was atomized from 0.7 mm nozzle into vertical, co-current drying chamber, 0.16 m diameter and 0.5 m height, using a full-factorial design to provide data and correlations that predict the powder properties as a function of the main operational variables of the spray-dryer. The inlet air (T_{inlet}) (140 and 200 °C) and feed (T_{feed}) (10 and 50 °C) temperatures, feed flow rate (V_{feed}) (2.1E-04 and 9.6E-04 kg s^{-1}) and atomizing air flow (V_{air-flow}) (1.3 E-0.4 and 1.9E-0.4 m^3 s^{-1}) were investigated as spray drying process variables.

Moisture Content

The moisture content (MC) of maltodextrin was measured with a halogen moisture analyzer (Ohaus, MB45, Switzerland) which was correlated well with the oven method, drying at 110 °C for 2 h.

Particle Properties

Particle size distribution: The particle size distribution of the maltodextrin was measured using a laser light diffraction particle size analyzer (MasterSizer model S 2000, Malvern Instruments Ltd., Worcestershire, U.K.) in which a small quantity of the powder was dispersed in water and the particle distribution was monitored during five successive trials. The particle size was expressed as mean area size D_{1,2} (Sauter mean diameter), and was calculated as follows:

\[
d_{1,2} = \sum \frac{n_i d_i^3}{\sum n_i d_i^2}
\]

(1)

Where \(n_i\) is the number of particles of diameter \(d_i\). The particle size distribution of the powder was measured as the span which is defined as:

\[
\text{span} = \frac{d_{90} - d_{10}}{d_{50}}
\]

(2)

Where \(d_{90}\), \(d_{10}\), and \(d_{50}\) are the equivalent volume diameters at 90%, 10%, and 50% cumulative volume, respectively.

Particle density: Particle density (\(\rho_p\)) of the powder samples was analyzed according to a study by Barbosa-Canovas et al. (2005). The liquid (petroleum ether) pycnometry was used to determine particle density depending on the volume of pycnometer bottle used.

Bulk Properties

Bulk and tapped densities: The bulk density (\(\rho_b\)) of the maltodextrin was determined by measuring the weight of the powder and the corresponding volume. Approximately 20 g of powder sample was placed in a 100 mL graduated cylinder. The bulk density was calculated by dividing the mass of the powder by the volume occupied in the cylinder. For the tapped density (\(\rho_t\)), the cylinder was tapped vigorously by hand until no further change in volume occurred (Jinapong et al., 2008).

Porosity: Porosity (\(\varepsilon\)) of the powder samples was calculated using the relationship between the tapped (\(\rho_t\)) and particle (\(\rho_p\)) densities of the powders as shown below (Jinapong et al., 2008):

\[
\varepsilon = \left( \frac{\rho_p - \rho_t}{\rho_p} \right) \times 100
\]

(3)

Statistical Analysis

All samples were analyzed in triplicate. The analysis of variance (ANOVA) at a confidence level of 95% was performed. All the results that were obtained were analyzed using Design Expert–version 7.0 software (Statease Inc., MI, USA).

Results and Discussion

D_{1,2} (Sauter mean diameter) and span values of maltodextrin with respect to the inlet air and feed temperatures, feed flow rate and atomizing air flow are given in Table 1.

<table>
<thead>
<tr>
<th>No</th>
<th>T_{feed}, °C</th>
<th>T_{inlet}, °C</th>
<th>V_{feed}, kg s^{-1}</th>
<th>V_{air-flow}, m^3 s^{-1}</th>
<th>D_{1,2}, µm</th>
<th>Span</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>140</td>
<td>2.1E-04</td>
<td>1.3 E-0.4</td>
<td>4.720</td>
<td>2.841</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>200</td>
<td>2.1E-04</td>
<td>1.3 E-0.4</td>
<td>4.644</td>
<td>2.119</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>140</td>
<td>2.1E-04</td>
<td>1.3 E-0.4</td>
<td>5.158</td>
<td>2.489</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td>200</td>
<td>2.1E-04</td>
<td>1.3 E-0.4</td>
<td>4.691</td>
<td>1.906</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>140</td>
<td>9.6E-04</td>
<td>1.3 E-0.4</td>
<td>5.797</td>
<td>3.439</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>200</td>
<td>9.6E-04</td>
<td>1.3 E-0.4</td>
<td>4.019</td>
<td>1.768</td>
</tr>
<tr>
<td>7</td>
<td>50</td>
<td>140</td>
<td>9.6E-04</td>
<td>1.3 E-0.4</td>
<td>5.029</td>
<td>2.640</td>
</tr>
<tr>
<td>8</td>
<td>50</td>
<td>200</td>
<td>9.6E-04</td>
<td>1.3 E-0.4</td>
<td>6.045</td>
<td>3.022</td>
</tr>
<tr>
<td>9</td>
<td>10</td>
<td>140</td>
<td>2.1E-04</td>
<td>1.9E-0.4</td>
<td>3.815</td>
<td>1.860</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>200</td>
<td>2.1E-04</td>
<td>1.9E-0.4</td>
<td>3.919</td>
<td>1.642</td>
</tr>
<tr>
<td>11</td>
<td>50</td>
<td>140</td>
<td>2.1E-04</td>
<td>1.9E-0.4</td>
<td>4.184</td>
<td>1.954</td>
</tr>
<tr>
<td>12</td>
<td>50</td>
<td>200</td>
<td>2.1E-04</td>
<td>1.9E-0.4</td>
<td>3.904</td>
<td>1.905</td>
</tr>
<tr>
<td>13</td>
<td>10</td>
<td>140</td>
<td>9.6E-04</td>
<td>1.9E-0.4</td>
<td>4.006</td>
<td>2.279</td>
</tr>
<tr>
<td>14</td>
<td>10</td>
<td>200</td>
<td>9.6E-04</td>
<td>1.9E-0.4</td>
<td>3.503</td>
<td>1.997</td>
</tr>
<tr>
<td>15</td>
<td>50</td>
<td>140</td>
<td>9.6E-04</td>
<td>1.9E-0.4</td>
<td>5.586</td>
<td>3.653</td>
</tr>
<tr>
<td>16</td>
<td>50</td>
<td>200</td>
<td>9.6E-04</td>
<td>1.9E-0.4</td>
<td>4.003</td>
<td>2.058</td>
</tr>
</tbody>
</table>

Spray-dried products are usually nonhomogeneous. For nonhomogeneous systems, with particle size distribution, the voids between big particles are filled with smaller particles, which cause an increase of the bulk density (Schubert, 1987). The Sauter mean
diameter \( (D_{3,2}) \) of a sphere, which gives an indication of the diameter corresponding to the average particle volume of the particle size distribution, and the span values varied between 1.642 and 3.653. The span-value expressing the width of the size distribution was also in the same order of magnitude. The particle size distribution of spray dried maltodextrin in 16 different experiments was shown in Figure 1-a and Figure 1-b. It can be seen that spray dried maltodextrin had a narrower particle size range with a relatively uniform distribution. The particle size distribution showed that all samples ranged from 3.503 and 6.045 µm. These results show that spray drying does not produce large particles. The mean particle size of a material may greatly influence its reactivity and the quality of the end product (Baranauskiene et al., 2006). Perturbation plots (Figure 2) showed that the Sauter mean diameter \( (D_{3,2}) \) of the maltodextrin powder was significantly affected by the atomizing air flow and inlet air temperature.

![Figure 1-a](image1.png)

**Figure 1-a.** Particle size distribution of spray dried maltodextrin under 16 different experimental spray drying conditions (first 8 experiments)

![Figure 1-b](image2.png)

**Figure 1-b.** Particle size distribution of spray dried maltodextrin under 16 different experimental spray drying conditions (last 8 experiments)

![Figure 2](image3.png)

**Figure 2.** Perturbation plot of Sauter mean diameter \( (D_{3,2}) \)

A – Inlet air temperature, B – Feed temperature, C – Feed flow rate, D – Atomizing air flow

<table>
<thead>
<tr>
<th>No</th>
<th>MC, %, wb</th>
<th>( \rho_b ), kg m(^{-3} )</th>
<th>( \rho_t ), kg m(^{-3} )</th>
<th>( \rho_p ), kg m(^{-3} )</th>
<th>( \varepsilon )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.857</td>
<td>287.9</td>
<td>510.5</td>
<td>1094</td>
<td>53.34</td>
</tr>
<tr>
<td>2</td>
<td>4.863</td>
<td>253.5</td>
<td>426.8</td>
<td>924.9</td>
<td>53.86</td>
</tr>
<tr>
<td>3</td>
<td>5.477</td>
<td>293.1</td>
<td>439.7</td>
<td>1088</td>
<td>59.57</td>
</tr>
<tr>
<td>4</td>
<td>4.600</td>
<td>262.6</td>
<td>409.9</td>
<td>1180</td>
<td>64.27</td>
</tr>
<tr>
<td>5</td>
<td>8.880</td>
<td>329.7</td>
<td>572.9</td>
<td>1061</td>
<td>45.98</td>
</tr>
<tr>
<td>6</td>
<td>4.267</td>
<td>254.0</td>
<td>402.4</td>
<td>1110</td>
<td>63.73</td>
</tr>
<tr>
<td>7</td>
<td>6.353</td>
<td>299.4</td>
<td>455.6</td>
<td>1304</td>
<td>65.04</td>
</tr>
<tr>
<td>8</td>
<td>4.633</td>
<td>331.6</td>
<td>538.9</td>
<td>1190</td>
<td>54.70</td>
</tr>
<tr>
<td>9</td>
<td>4.300</td>
<td>279.0</td>
<td>484.3</td>
<td>1034</td>
<td>53.14</td>
</tr>
<tr>
<td>10</td>
<td>3.813</td>
<td>245.2</td>
<td>417.6</td>
<td>993.8</td>
<td>57.98</td>
</tr>
<tr>
<td>11</td>
<td>11.13</td>
<td>297.8</td>
<td>489.5</td>
<td>1165</td>
<td>57.97</td>
</tr>
<tr>
<td>12</td>
<td>6.750</td>
<td>306.9</td>
<td>455.5</td>
<td>1077</td>
<td>56.72</td>
</tr>
<tr>
<td>13</td>
<td>6.743</td>
<td>313.0</td>
<td>536.5</td>
<td>1127</td>
<td>52.39</td>
</tr>
<tr>
<td>14</td>
<td>6.263</td>
<td>263.0</td>
<td>445.6</td>
<td>1089</td>
<td>59.09</td>
</tr>
<tr>
<td>15</td>
<td>10.91</td>
<td>349.1</td>
<td>518.4</td>
<td>1263</td>
<td>58.97</td>
</tr>
<tr>
<td>16</td>
<td>6.097</td>
<td>302.7</td>
<td>489.3</td>
<td>1057</td>
<td>53.72</td>
</tr>
</tbody>
</table>

The moisture content (%., wet basis), bulk and particle properties of spray dried maltodextrin obtained at
different experimental spray drying conditions is presented in Table 2. Moisture content values varied between 3.81 and 11.13%, wet basis. Maximum moisture content (11.13%, wb) value was recorded at inlet air temperature of 140 °C, feed temperature of 50 °C, feed flow rate 2.1E-04 kg s⁻¹ and atomizing air flow 1.9E-0.4 m³ s⁻¹ (Table 2). According to perturbation graphs (Figure 3), moisture content was affected significantly by the inlet air temperature.

The bulk properties (bulk and tapped densities and porosity) of a food powder are highly dependent on particle size and its distribution (Barbosa-Cánovas et al., 2005). Lower bulk densities of a product are not desirable, resulting in a greater volume of package. Moreover, lower the bulk density, more occluded air within the powders would be and a greater possibility for product oxidation resulting in reduced storage stability (Kurozawa et al., 2009). The bulk densities of samples were changed in the range of 245 and...
349 kg m\(^{-3}\) (Table 2). Bulk density and particle density were affected by all the independent variables except atomizing air flow (Figure 4 and 5) whereas tapped density was affected by only inlet air temperature and feed flow rate, respectively (Figure 6).

![Perturbation plot of porosity](image)

**Figure 7. Perturbation plot of porosity**

A – Inlet air temperature, B – Feed temperature, C – Feed flow rate, D – Atomizing air flow

Maximum porosity value (65.05) was recorded at inlet air temperature of 140 °C, feed temperature of 50 °C, feed flow rate 9.6E-04 kg s\(^{-1}\) and atomizing air flow 1.3 E-0.4 m\(^{3}\) s\(^{-1}\) (Table 2). Spherical particles were packed in the best and thus, have the highest bulk densities and porosity (Reineccius 2004). Porosity was influenced by the feed temperature and atomization air flow (Figure 7).

**Conclusions**

In this study, physical properties of maltodextrin powders were investigated. The results showed that spray drying does not produce larger particles. The bulk, tapped and particle densities of spray dried maltodextrin were (<350), (<575) and (<1310) kg m\(^{-3}\), respectively. These values are an indication that maltodextrin has fair porosity (65). For purposes of understanding the particle formation process, predicting product quality and evaluating the behavior of spray-dried maltodextrin during microencapsulation, this information will provide a helpful approach. Perturbation graphs revealed that atomizing air flow and inlet air temperature had more effect than feed temperature and feed flow rate on the physical properties of spray dried maltodextrin.

**References**

INTRODUCTION

Wild edible mushrooms have been widely used for centuries as a source of food and food-flavoring material in many countries. Mushrooms are valued due to their aroma and flavour (Guedes de Pinho et al., 2008a), rich content of carbohydrates and fibres (Mattila et al., 2000), vitamins and minerals, proteins and unsaturated fatty acids (Ribeiro et al., 2009). Edible mushrooms are often regarded as a food with antimicrobial, cholesterol-lowering, antioxidant and free radical scavenging properties (Ribeiro et al., 2006; Barros et al., 2007a; Aryantha et al., 2010). These mushrooms also have prophylactic properties against coronary heart disease and hypertension (Mattila et al., 2000). Various studies have been carried out on the chemical composition of the European edible wild mushrooms from several countries like Finland (Mattila et al., 2002), Poland (Bernas, Jaworska, 2010), Spain (Díez, Alvarez, 2001), Portugal (Barros et al., 2006, 2007a, 2007b, 2008), Italy (Manzi et al., 2001, 2004), Macedonia (Bauer-Petrovska, 2001), Greece (Ouzouni et al., 2007), Turkey (Ayaz et al., 2011) and Croatia (Beluhan, Ranogajec, 2011). Carbohydrates and crude proteins are the two main components, thus Cantharellus cibarius contains crude protein 53.7%, carbohydrates 31.9% and lipids 2.9% of the dry matter (Barros et al., 2008). Dry matter of mushrooms usually is in the range of 60–140 g kg⁻¹ (Kalač, 2009). Relatively low content of dry matter and lipids result in the low energy value of mushrooms (Barros et al., 2008). The analysis of the obtained profiles of fatty acids showed that oleic, linoleic and, to a lesser extent, palmitic and stearic acids were the main fatty acids in the studied Cantharellus cibarius (Ribeiro et al., 2009). The profile of organic acids consisted of citric, malic, fumaric, shikimic and ascorbic acids (Valentao et al., 2005). The content of free amino acids in mushrooms is low, only about 1% of the dry matter. The major amino acids found in Cantharellus cibarius were glutamic acid, lysine, alanine, and threonine (Beluhan, Ranogajec, 2011; Surinrat et al., 1987). Phenolic compounds have usually attracted attention due to their antioxidant properties. When the individual profile of phenolic compounds in edible mushrooms was studied, Boletus edulis was found to present the highest total content of phenolic compounds (Palacios et al., 2011) and total content of flavonoids (Palacios et al., 2011; Robaszkiewicz et al., 2010). Cantharellus cibarius was found to contain catechin, pyrogallol, myricetin, phenolic acids such as caffeic, ferulic, gallic, p-hydroxybenzoic, gentisic, homogentisic and protocatechuic acids. Content of caffeic acid and catechin in Cantharellus cibarius was higher than in other studied species, including Boletus edulis. Although the total content of phenolic compounds is lower, the total antioxidant activity in Cantharellus cibarius is higher than in any other mushroom studied, indicating that the increased content of caffeic acid and catechin is responsible for greater antioxidative power (Palacios et al., 2011). Valentao et al. (2005) have identified also the presence of 3-, 4- and 5-O-caffeoylquinic acids and rutin in Cantharellus cibarius. Mushrooms are also characterized by a high level of well assimilable mineral constituents. Potassium, magnesium, and phosphorus containing compounds are...
the most abundant in *Cantharellus cibarius* (Falandysz et al., 2012; Konuk et al., 2006). Portuguese scientists have investigated some volatile components in *Cantharellus cibarius* (Guedes de Pinho et al., 2008b), but no information is available on the changes in the content of volatile compounds after freeze-drying.

The aim of the present study was to investigate the chemical composition of widely used wild edible mushroom *Cantharellus cibarius* collected in Latvia.

**Materials and Methods**

Samples of *Cantharellus cibarius* were collected in Jelgava region (Latvia) in late summer 2011. After collection, mushrooms were freeze-dried (Christ Freeze Dryer Alpha 1-2 LD plus, Germany). All dried mushroom samples were grounded in a blender and then stored in air-tight bags at the room temperature until analysis.

Results of analysis were standardized by dry matter of samples. The ash content of mushrooms was determined by incineration at 550 °C (Manjunathan, Kaviyarasan, 2011). The protein amount in mushroom dry matter was determined by Lowry procedure (Lowry et al., 1951) using albumin as a standard.

Water extract was prepared as follows: 1 g of powdered mushrooms was boiled in 50 mL of water for 30 min. The mixture was centrifuged (3000 g, room temperature for 10 min) and supernatant portioned and kept frozen at -23 °C until analysis and used for determination of total phenolic content, formol number, titratable acidity, electrical conductivity. The total content of phenolic compounds in water extract was determined by Folin-Ciocalteu assay. Gallic acid (0–0.75 mg mL⁻¹) was used as a standard to produce the standard curve. The absorbance of the reaction mixture was measured at 765 nm using UV/Vis spectrophotometer Jenway UV 6405. The total content of phenolic compounds was expressed as milligrams of gallic acid equivalents (GAE) per gram of mushroom dry matter (Barros et al., 2007b).

In water extract titratable acidity was determined by potentiometric titration as described previously (Tanner, 1987) and calculated as mmol of NaOH per 1 g of mushroom dry matter.

The formol number was determined in water extract by potentiometric titration as described previously (Tanner, 1987). Formol number was calculated as mmol of NaOH per 1 g of mushroom dry matter.

Electrical conductivity in water extract was determined to characterise the total content of mineral substances using electrode TetraCon 325 connected to conductometer inoLab pH/Cond 720 (WTW, Germany).

Mushroom powder sample (1 g) was extracted with 50 mL of methanol at 25 °C for 24 h (Ribeiro et al., 2006; Barros et al., 2007a) and used for analysis of β-carotene and lycopene.

The content of the β-carotene and lycopene was determined spectrophotometrically. Obtained methanol extract was evaporated and 100 mg of the remaining dry matter were stirred with 10 mL of acetone-hexane mixture and filtered. The absorbance was measured at 453, 505 and 663 nm. The content of β-carotene (mg 100 mL⁻¹) and lycopene (mg 100 mL⁻¹) was calculated according to the following equations (Barros et al., 2007b):

\[ \text{Lyc} = -0.0458A_{663} + 0.372A_{505} - 0.0806A_{453}, \quad (1) \]

where \( \text{Lyc} \) = lycopene content, mg 100 mL⁻¹;

\[ A_{663} \] = absorbance at 663 nm;

\[ A_{505} \] = absorbance at 505 nm;

\[ A_{453} \] = absorbance at 453 nm.

\[ \beta\text{-carotene} = 0.216A_{663} - 0.304A_{505} + 0.452A_{453}, \quad (2) \]

where \( \beta\text{-carotene} \) = β-carotene content, mg 100 mL⁻¹.

The results were expressed as micrograms of carotenoid per gram of dry matter.

Volatiles from mushrooms were extracted using solid phase microextraction (SPME). 0.5 g of freeze dried mushrooms was weighed into a vial. Extraction and injection was performed manually. A 85 μm carboxen/ polydimethylsiloxane (Car/PDMS) fiber (Supelco Inc., Bellefonte, PA, USA) was used for headspace SPME sampling. SPME parameters were: equilibration time 10 min, extraction temperature 40±1 °C, extraction duration 30 min, desorption 15 min, 250 °C. For the analysis of the volatile compounds a Perkin Elmer Clarus 500 GC/MS equipped with a capillary column Elite-Wax ETR (60 m×0.25 mm i.d.; DF 0.25 μm) was used. Operating conditions were: injector temperature 250 °C; oven temperature start at 40 °C, hold 7 min, programmed from 40 to 160 °C at 6 °C min⁻¹, hold 10 min, and from 160 to 210 °C at 15 °C min⁻¹, hold 15 min; carrier gas (He) 2 mL min⁻¹; split 1 : 2; ionization El 70 eV; acquisition parameters in full scan mode: scanned m/z 50–300. Identification of compounds was achieved by comparing the mass spectra present in the NIST98 MS Library Database. The results are presented as the mean ± standard deviation of three measurements.

**Results and Discussion**

The content of dry matter of *Cantharellus cibarius* was 9.5±0.5% and was similar to our previous results of mushroom *Boletus edulis* (Kuka, Cakste, 2011). The content of dry matter of *Cantharellus cibarius* from Croatia was 14.2±0.2% (Beluhan, Ranogajec, 2011), while in study by Bernaś et al. (2006) the content of dry matter was found to be 7–12 g per 100 g of fresh matter and similar results were obtained in our study.

Ash amount was determined to characterize the content of mineral substances. Ash amount of *Cantharellus cibarius* was 7.7±0.02% of mushroom dry matter, more than 25% higher than for *Boletus edulis*. Similar tendency was observed when ash content was compared in *Cantharellus cibarius* (8.8±0.05 g 100 g⁻¹) and *Boletus edulis* (5.3±0.87 g 100 g⁻¹) mushrooms collected in Croatia (Beluhan, Ranogajec, 2011).
Electrical conductivity in water extract of Cantharellus cibarius was 2550±8 μS cm⁻¹. The obtained result also indicates that the amount of strong electrolytes in Cantharellus cibarius is high. Titratable acidity, that characterises the total amount of acids, is shown in Figure 1. Titratable acidity for Cantharellus cibarius was similar to the Boletus edulis f. pinicola and Boletus edulis f. beticola (2.26 and 2.22 mmol NaOH g⁻¹, respectively (Kuka, Cakste, 2011)). The formol number, which characterises the concentration of free amino acids, is shown in Figure 1. The concentration of free amino acids was almost negligible in Cantharellus cibarius, while the formol number for Boletus edulis f. beticola was 4.6 times higher (Kuka, Cakste, 2011).

The protein amount in mushroom Cantharellus cibarius was 190±2 mg g⁻¹, 1.7 times and 2.8 times lower than for Boletus edulis f. beticola and Boletus edulis f. pinicola, respectively (Kuka, Cakste, 2011). Content of protein in Cantharellus cibarius collected in Latvia was approximately 20%, while in several other studies the amount of protein was shown to vary from 10% (99 g kg⁻¹) (Danell, Eaker, 1992) to 53.7% of dry matter of Cantharellus cibarius (Kalač, 2009).

Figure 1. Titratable acidity (TA) and formol number (FN) expressed of Cantharellus cibarius dry matter

The content of total phenolic compounds was determined in water extract of Cantharellus cibarius and characterised using gallic acid equivalents (GAE) per 1 g of mushroom dry matter and was found to be 5.09±0.01 mg GAE g⁻¹ of mushroom dry matter. That is 2.2 times lower than for Boletus edulis f. beticola and 2.5 times lower than for Boletus edulis f. pinicola (Kuka, Cakste, 2011). Cantharellus cibarius from Poland and Portugal was shown to contain phenolic compounds 2.39±0.23 mg GAE g⁻¹ (Robaszkiewicz et al., 2010) and 1.75±0.50 mg GAE g⁻¹ (Barros et al., 2008) of dry matter, respectively, while Cantharellus cibarius from Latvia contained approximately 2-fold more phenolic compounds calculated as mg GAE g⁻¹.

The content of β-carotene and lycopene was determined using methanol extract of mushroom dry matter (Figure 2). The content of β-carotene was 4 times higher than the content of lycopene for Cantharellus cibarius, but 4.6 times less than for Boletus edulis f. beticola (Kuka, Cakste, 2011). Cantharellus cibarius collected in Portugal contained 5.77±0.42 μg g⁻¹ of β-carotene and 1.95±0.28 μg g⁻¹ of lycopene (Barros et al., 2008) and comparable amounts of β-carotene and lycopene were also found in mushrooms collected in Latvia.

Figure 2. The content of β-carotene and lycopene of mushroom Cantharellus cibarius dry matter

Using gas chromatography-mass spectrometry (GC-MS) volatile compounds were identified in both fresh and freeze-dried mushrooms Cantharellus cibarius. Oct-1-en-3-ol, (Z)-oct-2-en-1-ol, hexanal, hept-1-en-3-one, ethyldeca-2,4-dienoate, (E)-oct-2-enal, octan-3-one were identified in fresh mushroom (Figure 3). The dominant compound was found to be oct-1-en-3-ol. The study of Du et al. (2010) similarly to the current research revealed that most abundant aroma compounds are alcohols, aldehydes, and ketones. Among other important compounds were identified terpenes, eight carbon compounds and their derivatives. According to the research of Cheng et al. (2012) oct-1-en-3-ol gives a unique earthy taste and sweetness to mushroom aroma. As show previous studies alcohols and ketones, mainly C8 aliphatic compounds, like octan-3-ol, oct-2-en-1-ol, octan-1-ol and octan-3-one were the main compounds responsible for characteristic mushroom-like aroma emitted by some of mushrooms (Malheiro et al., 2013).

In the current study great losses of volatile compounds were found in freeze-dried mushrooms. The total sum of peak areas in fresh Cantharellus cibarius mushroom was 166.08×10⁶, which decreased as a result of drying till 93.8×10⁶. Content of oct-1-en-3-ol was decreased more than 5 times suggesting significant change of mushroom-like aroma. The loss of the noted alcohol could be due to is high volatility. (Z)-oct-2-en-1-ol, hept-1-en-3-one, ethyldeca-2,4-dienoate, (E)-oct-2-enal and octan-3-one were not identified in freeze-dried samples.
Conclusions
Although the amount of substances tested is slightly higher in mushroom Boletus edulis, mushroom Cantharellus cibarius is a rich source of biocompounds and mineral substances. Freeze-drying significantly reduces the amount of volatile compounds and could affect flavour of mushrooms. Together, Latvian Cantharellus cibarius have similar dry matter, ash and protein content, amount of β-carotene and lycopene, but two fold higher amount of phenolic compounds than Cantharellus cibarius collected in other regions of Europe.

References

Figure 3. Chromatogram of volatile compounds of fresh mushroom Cantharellus cibarius
SOLVENT EXTRACTION OF EGG OIL FROM LIQUID EGG YOLK

Aleksandrs Kovalciks, Mara Duma
Department of Chemistry, Faculty of Food Technology, Latvia University of Agriculture, Liela street 2, Jelgava, Latvia, e-mail: aleksandrskovalciks@inbox.lv

Abstract
The egg yolk lipids have a very high nutritional value. Due to the fatty acid profile, high oil soluble vitamin and lecithin content egg yolk oil can be used as a very good additive to a human nutrition. There are several methods for oil extraction from egg yolks known, but in this manuscript solvent extraction of egg yolk oil from the liquid egg yolk was studied as a most economically reasonable method. The aim of this study was to compare two different solvent mixtures ethanol/chloroform (30/70 by volume) and 2-propanol/hexane (30/70 by volume) for oil extraction from liquid egg yolk and determine quality and nutritional properties of extracted oils. As a liquid egg yolk was used for extraction of oil, the choice of the solvents was based on solvents polarities. Also volatility and toxicity of the solvents were taken in account. The yield of extracted crude oil, water content, solvent residue, fatty acid profile and β-carotene content were determined and compared. The results show that extraction with 2-propanol/hexane gave higher yield of the crude oil than ethanol/chloroform, 28.90±0.27% and 26.37±1.04%, respectively. High water content and solvent residue in both oils mean that there is a purification of solvent extracted egg yolk oil needed. There was no significant difference in fatty acid profile in both oils, but β-carotene content was higher in oil extracted with 2-propanol/hexane 81.02±0.37 mg kg⁻¹, than in ethanol/chloroform extract 73.16±1.53 mg kg⁻¹.

Keywords: egg oil, solvent extraction method.

Introduction
The egg yolk lipids have a very high nutritional value. Thanks to the fatty acid profile, high oil soluble vitamin and lecithin content egg yolk oil can be used as a very good additive to a human nutrition (Lewis et al., 2000). Nutritional properties in eggs can be affected through the laying hen feed (Surai et al., 2008; Stibilj et al., 1999). There are good ways known how to enrich egg yolk oil with nutrients, but how to extract them as much as possible and keep them in their natural condition after extraction remains the question. There are several methods for oil extraction from egg yolks known, but in this manuscript solvent extraction of egg yolk oil from the liquid egg yolk was studied as a most economically reasonable method. In case of solvent extraction high technological and safety requirements must be taken in account when choosing solvents for extraction. Solvents must extract as much as possible lipids from raw material, must have a low boiling temperature, for easier and economical removing from the product, and must be as less as possible toxic.

The difference of oil extraction from egg or egg yolk powder is that liquid egg yolk contains a lot of water and extraction with non-polar solvents is not efficient due to difference in solvent and egg yolk polarities. Polar solvents, such as lower alcohols, denature egg yolk proteins destroying hydrogen bonds or electrostatic interaction in protein structure opening the way to the neutral lipids, what makes extraction with non-polar solvent possible. Without protein denaturation, polar solvents will extract polar membrane-associated lipids from the egg yolk. The combination of polar and non-polar solvents for better lipid extraction from liquid egg yolk can be chosen. The 2-propanol/hexane mixture can be a good solvent for extraction of egg oil from liquid egg yolk (Ahn et al., 2006).

2-propanol / hexane solvent
2-propanol, a quite polar solvent, can denature egg yolk proteins and extract polar lipids, but it is also non-polar enough to be soluble in non-polar solvent as hexane. 2-propanol is relatively low toxic and is accepted as a solvent in the processing of foodstuff (EFSA, 2005). Main disadvantages of 2-propanol is its boiling temperature which is 82.6 °C, but boiling temperature can be decreased by using vacuum in evaporation procedure. Hexane is a most popular solvent for lipid extraction for food application. It is widely used in production of vegetable oils. In comparison with 2-propanol, hexane will extract mainly simple triglycerides – neutral lipids. Hexane boiling temperature is 69 °C, which makes it very economical in solvent extracted oil production. Taking in account all before mentioned advantages the choice of non-polar solvent was quite simple. Because two solvents was mixed together to make extraction mixture, the ratio 30:70 between 2-propanol and hexane was chosen. Too much of 2-propanol will make mixture too polar to extract neutral lipids as much as possible.

Ethanol/chloroform solvent
One of the main tasks of oil extraction is the high yield of oil. The process where total lipid extraction is needed is an analysis of the fat content in food products. For fat analysis in egg containing food products methanol/chloroform is used as a solvent (Boselli et al., 2001). Methanol usage for food grade oil extraction is not safe due to the toxicity of this solvent. For that reason methanol was replaced with ethanol. Ethanol is a lower alcohol, same as 2-propanol, it denatures egg yolk proteins and as a polar solvent extracts polar lipids. Chloroform is widely used in defatting of biological materials. The process of lipid extraction from tissues with methanol/chloroform first was mentioned in 1957 (Folch et al., 1957). The so-called Folch procedure requires addition of water to solvent mixture, but in

FOODBALT 2014
case of extraction of liquid egg yolk, water is already present in the egg yolk. Chloroform / methanol mixture still is used in laboratories for fat content determination in food products (Cizkova et al., 2004). But it is must be admitted that chloroform is toxic and even suspected of causing cancer (WHO, 2004). The aim of this study was to compare two different solvent mixtures – ethanol/chloroform (30/70 by volume) and 2-propanol / hexane (30/70 by volume) for oil extraction from liquid egg yolk and determine quality and nutritional properties of extracted oils. The quality of egg yolk oil during the storage can be decreased by lipid oxidation. In this case water content in the oil can affect oxidation process. Egg yolk contains approximately 50% of water and extraction of egg yolk lipids with polar solvents, without extraction of polar lipids, can also bring some water to the extract. After oil extraction with organic solvents they must be removed from the final product. The solvent residue in extracted oil is an important quality parameter. All solvents used in food processing are strictly controlled by food safety regulators on its presence in food products (Directive 2009/32/EC, 2009). The main nutritional value of egg yolk oil is unsaturated fatty acids. The extraction method can be evaluated as acceptable if it does not affect the fatty acid profile of egg oil. β-carotene, as a vitamin A precursor, is a representative of oil soluble vitamins in egg yolk. Content of β-carotene in egg yolk oil can reflect the efficiency of extraction process. The very attractive colour of egg yolk oil is related to β-carotene and therefore its concentration can be easily detected by a simple photometric method.

Materials and Methods

Materials

Commercially available homogenized, pasteurized and chilled liquid egg yolk was obtained from JSC Balticovo (Iecava area, Latvia). Liquid yolk was stored at +4 °C and used with the same temperature for extraction. All solvents (ethanol, 2-propanol, chloroform, hexane) used in egg yolk oil extraction were with the analytical grade from Sigma Aldrich, Germany.

Egg yolk oil extraction

For egg yolk oil extraction two solvent mixtures - ethanol/chloroform (30/70) and 2-propanol/hexane (30/70) were used. For each extraction process solvents were mixed by volume and poured in beaker. Liquid egg yolk was added to solvent mixture with a thin squirt vigorously mixing. The ratio 2 : 1 between solvent mixture and egg yolk was used. Extraction was done at +21 °C temperature vigorously mixing for 30 minutes. Extracts were filtered using vacuum filtration and collected into a clean container. The oil was recovered by evaporation of the solvent mixture using rotary evaporator IKA R 10 Control (IKA-Werke GmbH & Co. KG) at +70 °C for ethanol / chloroform extract and +80 °C for 2-propanol / hexane extract under the vacuum.

Analyses

The yield of egg oil after extraction with different solvent mixtures was expressed as a weight of total lipids extracted from liquid egg yolk in percent. Fatty acid profile was determined in accordance with standard methods ISO 12966-2 and ISO 5508, GC-FID (gas chromatography with flame ionization detector). Shimadzu GC 2010 Plus gas chromatograph with flame ionization detector (Shimadzu Corporation, Japan) was used.

Water content in egg yolk oil was measured with Moisture balance MOC-120H scale from Shimadzu (Shimadzu Corporation, Japan). Water evaporated at 120°C until difference in the sample weight was less than 0.05 percent.

β-carotene content was determined in accordance with standard method ISO 17932:2012 using UV spectrophotometer UV-1800 (Shimadzu Corporation, Japan).

Solvent residue in both oil samples was detected by combination of headspace and GC-FID (gas chromatography with flame ionization detector) methods (Restek, 2000; ISO 9832:2002; Stenerson, Verma, 2011; Tiscione et al., 2011).

The results are the means and standard deviation for three replicates. Means were compared by T-test and analysis of variance (ANOVA). Significance was defined at p<0.05. Statistical analysis was carried out by Microsoft Excel 2010 version software.

Results and Discussion

Extraction process

It needs to be admitted that in the extraction process the particle size of denatured egg yolk proteins was different. In ethanol/chloroform extraction protein fraction had smaller aggregates and, due to the high density of chloroform, protein fraction was positioned in the top layer. Vacuum filtration of ethanol/chloroform extract requires a longer period of time comparing to 2-propanol / hexane. In case of 2-propanole / hexane extraction, yolk proteins had a bigger particle size and proteins were deposited in bottom layer. This information is important for choosing filtration equipment for egg yolk oil in large scale production.

The results presented in Table 1 show quality parameters of both extracted egg yolk oils. The yield of crude egg yolk oil extracted from liquid egg yolk with 2-propanol / hexane solvent was significantly higher than from ethanol / chloroform extraction (p<0.05). The determined water content was high in both oils. This can be explained by the fact that liquid egg yolk contains approximately 50% of water and extraction with mixture of polar and non-polar solvent causes the phase separation where egg yolk phospholipids dissolve in polar phase and start to swell absorbing the water from the egg yolk. The presence of water in egg yolk oil can affect quality of egg oil during storage.
resulting in rancidity. Egg oil needs to be purified to increase its storage time. After solvent removal from the extracts by means of evaporation under the vacuum, crude egg yolk oil was analyzed for solvent residues (Table 1). Ethanol, 2-propanol and hexane, can be used as extraction solvents in food processing in compliance with good manufacturing practice, but anyway these solvent residues in food product are specified by a maximum residue limits in extracted foodstuff or food ingredient, where 2-propanol is limited to 10 mg kg\(^{-1}\) and hexane to 1 mg kg\(^{-1}\) (Directive 2009/32/EC, 2009; EFSA, 2005). The residue of 2-propanol and hexane in egg oil exceeds the limits allowed in food products and means that the solvent recovery process must be optimized for better solvent removal. The allowed daily intake of ethanol varies, but its residue in ethanol/chloroform extracted egg yolk oil must not exceed the dangerous limits for human health. Chloroform is a very toxic chemical and its presence in food products even in a very small concentration can have influence on human health therefore chloroform containing food is prohibited under any circumstance in most countries.

<table>
<thead>
<tr>
<th>Quality parameters</th>
<th>Extraction solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield, %</td>
<td>28.90±0.27</td>
</tr>
<tr>
<td>Water content, %</td>
<td>14.75±1.05</td>
</tr>
<tr>
<td>2-propanol, mg kg(^{-1})</td>
<td>264.14±7.18</td>
</tr>
<tr>
<td>Hexane, mg kg(^{-1})</td>
<td>2.03±0.02</td>
</tr>
<tr>
<td>Ethanol, mg kg(^{-1})</td>
<td>–</td>
</tr>
<tr>
<td>Chloroform, mg kg(^{-1})</td>
<td>86.08±12.38</td>
</tr>
</tbody>
</table>

\(\beta\)-carotene content
\(\beta\)-carotene content in egg yolk oil extracted with 2-propanol/hexane solvent was 81.02±0.37 mg kg\(^{-1}\) and 73.16±1.53 mg kg\(^{-1}\) in ethanol / chloroform extracted oil. The higher content of \(\beta\)-carotene in 2-propanol / hexane extracted egg yolk oil can be explained by usage of particularly non-polar solvent hexane. \(\beta\)-carotene is a non-polar compound and it dissolves better in non-polar solvents such hexane. Hexane is usually used for extraction of \(\beta\)-carotene from carotenoid containing products.

Fatty acid profile
The fatty acid profile of 2-propanol / hexane and ethanol / chloroform extracted egg yolk oils presented in Table 2. The results show that there is no difference in fatty acid content for both oils. Both 2-propanol / hexane and ethanol/chloroform gave the same fatty acid profile of egg yolk oil.

<table>
<thead>
<tr>
<th>Fatty acid profile of egg yolk oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extraction solvent</td>
</tr>
<tr>
<td>Fatty Acid</td>
</tr>
<tr>
<td>2-propanol / hexane</td>
</tr>
<tr>
<td>g 100 g(^{-1})</td>
</tr>
<tr>
<td>(C14:0)</td>
</tr>
<tr>
<td>(C14:1)</td>
</tr>
<tr>
<td>(C15:0)</td>
</tr>
<tr>
<td>(C16:0)</td>
</tr>
<tr>
<td>(C16:1)</td>
</tr>
<tr>
<td>(C17:0)</td>
</tr>
<tr>
<td>(C17:1)</td>
</tr>
<tr>
<td>(C18:0)</td>
</tr>
<tr>
<td>(C18:1)</td>
</tr>
<tr>
<td>(C18:2)</td>
</tr>
<tr>
<td>(C18:3)</td>
</tr>
<tr>
<td>(C20:1)</td>
</tr>
<tr>
<td>(C20:2)</td>
</tr>
<tr>
<td>(C20:3)</td>
</tr>
<tr>
<td>(C22:1)</td>
</tr>
<tr>
<td>(C20:4)</td>
</tr>
<tr>
<td>(C20:5)</td>
</tr>
<tr>
<td>(C24:1)</td>
</tr>
<tr>
<td>(C22:4)</td>
</tr>
<tr>
<td>(C22:5)</td>
</tr>
<tr>
<td>(C22:6)</td>
</tr>
<tr>
<td>Other</td>
</tr>
<tr>
<td>SFA</td>
</tr>
<tr>
<td>MUFA</td>
</tr>
<tr>
<td>PUFA</td>
</tr>
</tbody>
</table>

Egg yolk for an egg yolk oil extraction was obtained from laying hens which were fed with supplementation of canola oil and can be considered as a product enriched with unsaturated fatty acids. In comparison with other researchers (Stibilj et al., 1999; Souza et al., 2008) our extracted oil contains more oleic acid, but less palmitic and linoleic acids. But the difference in fatty acid content must be related more to the hen feed than to the extraction method or used solvents. Anyway extracted egg yolk oil due to the high content of monounsaturated and polyunsaturated fatty acids is a high value product which after purification can be used as an additive to a human nutrition providing the health benefits.

Figure 1 shows the content of main fatty acids yolk and total content of saturated, monounsaturated and polyunsaturated fatty acids in egg yolk oil and egg.
Figure 1. Fatty acid content in egg yolk and egg yolk oils extracted with 2-propanol / hexane and ethanol / chloroform

Conclusions

Solvent extraction of egg yolk oil from liquid egg yolk can be applied with combination of a polar solvent, as lower alcohols, and a non-polar solvent. As a result egg yolk oil rich in monounsaturated and polyunsaturated fatty acids and β-carotene can be obtained. Better extraction results were achieved with 2-propanol / hexane solvent which gave higher yield of oil and β-carotene content. Extraction of egg yolk oil from liquid egg yolk with ethanol / chloroform also is possible, but for the health safety reasons usage of chloroform must be prohibited. Both 2-propanol / hexane and ethanol / chloroform extracted oils have the same fatty acid profile. Due to the high water content and solvent residue crude egg yolk oil must be purified.

References

8. ISO 17932 (2011) Palm oil – determination of the deterioration of bleachability index (DOBI) and carotene content.
STORAGE STABILITY OF MICROENCAPSULATED EXTRA VIRGIN OLIVE OIL POWDER

Aslı Zungur¹, Mehmet Koç², Buket Yalçın³, Figen Kaymak-Ertekin¹, Semih Ötleş¹

¹ Department of Food Engineering, Faculty of Engineering, Ege University, Bornova, Izmir, Turkey
e-mail: figen.ertekeন@ege.edu.tr
² Department of Food Engineering, Faculty of Engineering, Adnan Menderes University, Aydın, Turkey

Abstract

Microencapsulation is the process that aims to increase the stability of food components (oil, pigments, aroma, etc.) during the processing and storage. Microencapsulation is used to convert extra virgin olive oil to powder form maintaining high oxidation stability, phenolic content and antioxidant capacity along the storage. In this study, the storage stability of microencapsulated extra virgin olive oil powder coated with maltodextrin and whey protein isolate as wall materials was investigated by evaluating microencapsulation efficiency, peroxide value, phenolic content and antioxidant activity during storage in aluminium polyethylene pouches at 25 °C and 50% relative humidity (RH) for 180 days. The results showed that microencapsulation efficiency (ME), phenolic content and antioxidant activity of microencapsulated extra virgin olive oil powder (MEVOP) during storage decreased 16%, 44.6% and 58.6%, respectively as compared to initial value, whereas the peroxide value of sample increased from 11.6 meq O₂ kg⁻¹ oil to 35.6 meq O₂ kg⁻¹ oil in the 180 days of storage. During storage, the degradation of phenolic content, antioxidant activity and the increase in the peroxide value of microencapsulated extra virgin olive oil were found to fit the first-order kinetic model. Although microencapsulation efficiency of samples changed slightly, phenolic content and antioxidant activity drastically reduced and peroxide value of samples increased sharply during the storage. This is possibly caused by small particle size and/or huge surface area of olive oil powder.

Keywords: microencapsulation, extra virgin olive oil, storage, oxidation.

Introduction

Extra virgin olive oil is obtained mechanically from ripe fruit of the olive tree without any chemical treatment. Extra virgin olive oil is an important vegetable oil with unique taste and odour which can be consumed naturally (Göğüs et al., 2009). Among the different categories of olive oil, the extra virgin olive oil is outstanding in gastronomic, nutritional, therapeutic and economic importance (Mendez, Falque, 2007). Studies conducted by other researchers attributed these benefits were due to its mono unsaturated fatty acid content (Fernández-Jarde et al., 2002; Martínez-González et al., 2002; Tripoli et al., 2005). However, recent research indicates that minor constituents appear to prevent several diseases. The phenolic fraction has importance regarding to antioxidant activities (Bendini et al., 2010) and the changes in the phenolic compounds over time could be an important quality control parameter of extra virgin olive oil.

The environmental conditions including oxygen, light, moisture and temperature make olive oil susceptible to oxidation due to high amount of monounsaturated fatty acid (oleic acid) content (Calvo et al., 2010; O’Brien, 2004). Oxidation is the main cause of olive oil quality deterioration and its reaction rate determines the shelf life of oil (Gomez-Alonzo et al., 2007). Oxidation that occurs in edible oils relates to the loss of minor components and formation of new compounds, causing nutritional loss as well as the development of rancid and other off-flavours (Velasco et al., 2003; Sun-Waterhouse et al., 2011, Cicerale et al., 2013).

Storage conditions are considered as critical variables that affect the quality of olive oil and its shelf life which is attributed to lipid oxidation mechanism which leads to rancidity (Vacca et al., 2006). Because olive oil is produced in a limited period of time, but consumed throughout the year, it must be stored, and this storage period determines the commercial life of the olive oil (Hrncirik, Fritsche, 2005; Kritsakis, Dugan, 1984; Zanoni et al., 2005). Many studies on oxidation of virgin olive oils were based on accelerated test measurements (Apicario et al., 1999; Baldioli, 1996; Gutierrez et al., 2002; Krichene et al., 2010). In these studies, good correlations between changes in various components and stability were found.

Microencapsulation can be used to protect fragrances or other active agents of olive oil from oxidation caused by heat, light, moisture, from contact with other substances over a long shelf life, to prevent evaporation of volatile compounds (Ghosh, 2006; Soest, 2007). Microencapsulation is a process of coating individual particles or droplets with a continuous film to produce capsules in a micrometer to millimeter in size (Tyagi et al., 2011). Microencapsulation process provides protection of reactive substances from the environment, converting liquid active component into a dry solid system and separation incompatible components for functional reasons. The surface oil content or microencapsulation efficiency of microencapsulated oil powder determines the storage stability. While converting olive oil to powder form, the surface area of olive oil expands that makes it more sensitive to oxidation.

The aim of this study was to evaluate the storage stability of microencapsulated extra virgin olive oil powder by analysing microencapsulation efficiency, peroxide value, total phenol content and antioxidant activity during 180 days storage.

Materials and Methods

Material

Extra virgin olive oil (ρ=910 kg m⁻³, µ=0.083 Pa·s at 20 °C) was purchased from a local grocery store in...
Turkish. The whey protein isolate (WPI) supplied by Ak Gida San. Tic. A.Ş. and maltodextrin (MD, DE19) provided by Qinhuangdao StarCh Co Ltd., were used as wall materials in this study. Tween 20 (Merck, Darmstadt, Germany) was used to stabilize the olive oil-water emulsion.

Preparation of olive oil in water emulsion

Maltodextrin (128.8 g) was dissolved in water (200 g) and kept in the dark for one night. Whey protein isolate (9.8 g) was mixed with water (100 g) using mechanical homogenizer (Ultra Turrax T25) at 0.167 kHz for 60 s. Tween 20 was added to olive oil in concentration 1% (w/w) as a stabilizer. Mixture of olive oil and Tween 20 was poured drop wise into the WPI solution using the mechanical homogenizer at 0.167 kHz for 60 s for pre-mixing. In order to obtain stable olive oil in water emulsions (o/w), WPI, olive oil and Tween 20 containing solution was mixed with MD solution and the whole mixture were homogenized using a mechanical homogenizer (Ultra Turrax T25) at 0.291 kHz for 300 s. Prepared emulsion contains 92% (db) maltodextrin and 7% (db) whey protein isolate as wall material. The total solids concentration of feeding emulsion is 40%. The olive oil concentration of the emulsion is 30% in dry basis.

Microencapsulation of olive oil by spray drying and storage of powder

The prepared emulsions were dried in a laboratory scale spray drier (Buchi, B-290, Switzerland) equipment with 0.7 mm diameter nozzle. The spray dryer conditions; the inlet air temperature 200°C, pump rate 22%, aspiration ratio 0.0067 m$^3$ s$^{-1}$ and air flow rate 0.00013 m$^3$ s$^{-1}$ were kept constant during the experiments. The feeding emulsions at 25 °C were fed to spray dryer and the obtained powder was packed in hermetically sealed aluminium laminated polyethylene (ALPE) pouches.

Microencapsulated olive oil powders were stored at 25 °C and 50% RH for 180 days. In the first 90 days of storage period, the analyses were be carried out in 15 days periods and then 30 days period was followed.

Total oil content

Total oil content of samples was determined according to method of Folch et al. (1957) with some modification. The microencapsulated extra virgin olive oil powder (4 g) was put into an extraction thimble that contained chloroform/methanol (80 mL; 3 : 2 v/v). The obtained mixture was homogenized at 0.0026 kHz for 900 s. Then water (16 mL) was added, and the mixture was shaken vigorously to facilitate the transfer of oil into the chloroform and other products into the water-methanol layer. The chloroform layer was then separated via a separation funnel and collected. The extraction steps were repeated two times. The obtained chloroform layers were combined and evaporated using a rotary evaporator at 65 °C, for 20–30 min. Then the trace amount of chloroform was evaporated in drying oven (at 102 °C temperature for 4 hours). After oven drying remaining is the amount of total oil of MEVOP.

Surface oil content

To determine surface oil, 5 g encapsulated beads were weighed into flaks and 25 mL petroleum ether was added. The mixture was extracted for 5 min at room temperature in the dark. The mixture was filtered and petroleum ether was evaporated. Then trace amount of petroleum ether was evaporated in drying oven (at 102 °C temperature for 4 hours) (Folch et al., 1957). After oven drying remaining is the amount of surface oil of MEVOP.

Microencapsulation efficiency (ME)

Microencapsulation efficiency of extra virgin olive oil powder was calculated using the following formula:

\[
ME = \frac{\text{Total oil content} - \text{Surface oil content}}{\text{Total oil content}} \times 100
\]  

Peroxide value

The peroxide value of extracted olive oil was expressed as peroxide milliequivalent per kg oil. Extracted olive oil (0.5 g) was dissolved in acetic acid-chloroform solution (3 mL; 3 : 2 v/v). After saturated potassium iodide KI solution was added, the mixture was left to stand for 1 min with occasional shaking. An aliquot (3 mL) of Milli-Q water was added. The mixture was titrated with 0.01 N standardized sodium thiosulphate solution until the yellow iodine colour just disappeared. Starch indicator solution (0.2 mL, 10 kg m$^{-3}$) was added. The titration continued until the blue colour derived from the iodine just disappeared. A blank sample as reagent control was set up and carried through all the steps (AOCS, 1998).

Phenol Extraction from Olive Oil and Total Phenol Analysis

For phenolic components analysis, firstly the cartridge (Maxi-Clean™ SPE 300mg Hi-Cap C18 cartridge) was conditioned by using dietylether / n-hexane (98 : 2 ratio). Then, 5 g extracted olive oil from microcapsules was weighed and 5 mL hexane was added. This mixture was filtered through the cartridge and cartridge was washed by using 2 mL hexane. Finally the 1.5 mL methanol was filtered from cartridge and the phenolic components were extracted from olive oil (Pellegrini et al., 2001). From the last extract, 50 µL sample was taken and 250 µL Folin-Ciocalteau reactive was added. After 5 min of holdingin dark at room temperature, 750 µL 7% Na$_2$CO$_3$ solution was added and the mixture was completed to 5mL by using distilled water. After 2 hours waited at room temperature in dark, the absorbance of the extracts was determined at 765 nm by using spectrometer. All these steps were done for blank. The standard curve was plotted by using caffeic acid as a standard (Singleton, Rossi, 1965; Singleton et al., 1999).

Total Antioxidant Activity Measurement-ABTS Method

Firstly ABTS radical was prepared as, 7mM ABTS water solutions and 2.45 mM potassium persulphate (1 : 0.5 ratio, v : v) were mixed and diluted by ethanol till the absorbance (730 nm) was 0.70. Then, 1 mL
ABTS radical was added into 20µL sample (0.1 g oil 1 mL chloroform). The absorbance was read at 730 nm. Finally, standard curve was drawn by using Trolox (1 mM to 5 mM). The results are given as Trolox equivalent (Pellegrini et al., 2001).

**Kinetic analysis of the given quality parameters during storage**

The obtained data were subjected to the kinetic modelling in order to determine the degradation kinetics of some quality characteristics (peroxide value, phenolic content and antioxidant activity) during storage. Reaction rate and the relevant coefficients were determined with Eq. (2) using SPSS version 13.0 Windows program (SPSS Inc., Chicago, IL).

\[ -\frac{dc}{dt} = k \times C \]  

(2)

where: C is the given quality parameter values, t is time and the k is the kinetic constant.

**Results and Discussion**

The amount of surface oil content of encapsulated product is important for ensuring the storage stability (Anandaraman, Reineccius, 1987). The microencapsulated products, which have high surface oil content, could be more sensitive to oxidation. The change in total oil content, surface oil content and microencapsulation efficiency of microencapsulated extra virgin olive oil powder during storage were given in Table 1. Along the storage of microencapsulated extra virgin olive oil powder, surface oil content increased without a change in total oil content. Therefore, microencapsulation efficiency decreased with increasing storage time. This indicates that, microcapsules were broken and oil in the capsules moves to the surface.

**Peroxide, total phenolic content and antioxidant activity values of MEVOP during storage**

<table>
<thead>
<tr>
<th>Time, days</th>
<th>Total oil, %</th>
<th>Surface oil, %</th>
<th>ME, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>27.28±0.22</td>
<td>3.63±0.30</td>
<td>86.69±1.91</td>
</tr>
<tr>
<td>15</td>
<td>26.45±0.40</td>
<td>4.19±0.11</td>
<td>84.15±1.91</td>
</tr>
<tr>
<td>30</td>
<td>27.05±0.41</td>
<td>4.70±0.56</td>
<td>82.63±1.76</td>
</tr>
<tr>
<td>45</td>
<td>27.54±0.47</td>
<td>5.10±0.25</td>
<td>81.45±1.18</td>
</tr>
<tr>
<td>60</td>
<td>28.02±0.34</td>
<td>5.36±0.71</td>
<td>80.84±0.82</td>
</tr>
<tr>
<td>75</td>
<td>27.34±0.27</td>
<td>5.44±0.13</td>
<td>80.12±1.09</td>
</tr>
<tr>
<td>90</td>
<td>28.39±0.30</td>
<td>5.73±0.14</td>
<td>79.83±0.70</td>
</tr>
<tr>
<td>120</td>
<td>27.11±0.50</td>
<td>6.68±0.68</td>
<td>75.35±2.41</td>
</tr>
<tr>
<td>150</td>
<td>27.97±0.88</td>
<td>8.15±0.54</td>
<td>73.49±1.59</td>
</tr>
<tr>
<td>180</td>
<td>28.39±0.50</td>
<td>6.77±0.99</td>
<td>72.80±3.46</td>
</tr>
</tbody>
</table>

Extra virgin olive oil is the only common food rich in a potent antioxidant called hydroxytyrosol. Antioxidants in the olive oil absorb free radicals and appear to have a positive impact on cardiovascular and cancer ailments, as attributed to the Mediterranean diet. For this reason, antioxidant capacity of olive oil must be protected during the conversion to the powder form and the storage period. Antioxidant activity decreased slightly in the early months of storage, but after the second months of storage decrease in the antioxidant activity has accelerated.
During storage, the degradation kinetics of phenolic compounds and antioxidant activity and the oxidation kinetics using peroxide value of microencapsulated extra virgin olive oil powder were determined using the first-order reaction kinetic model (Eq 2). Estimated reaction rate constants and $R^2$ values were given in Table 3.

### Conclusions

The present work showed that microencapsulation efficiency of the extra virgin olive oil powder decreased with respect to storage time. Peroxide value of sample increased from 11.6 meq O$_2$ kg$^{-1}$ to 35.6 meq O$_2$ kg$^{-1}$ at the end of storage period. Natural or synthetic antioxidants could be used to prevent lipid oxidation. During 180 days of storage period at 25 °C, antioxidant activity and total phenolic values of microencapsulated extra virgin olive oil powder decreased 58.6% and 44.6%, respectively. First order reaction kinetic model was found to be suitable to describe the degradation kinetics for total phenol content and antioxidant activity of samples and the increase in peroxide value of samples.

### Acknowledgment

The authors acknowledge for the financial support to TUBITAK-TOVAG (Project number: 111 O 345) and Ege University Science and Technology Centre (Project Number: 12/BIL/018).

### References


LEAF VEGETABLES AS SOURCE OF PHYTOCHEMICALS

Mara Duma¹, Ina Alsina², Solvita Zeipina²,³, Liga Leps³, Laila Dubova²

¹ Department of Chemistry, Faculty of Food Technology, Latvia University of Agriculture, Liela Street 2, Jelgava, Latvia
² Institute of Soil and Plant Sciences, Faculty of Agriculture, Latvia University of Agriculture, Liela Street 2, Jelgava, Latvia
³ Pure Horticultural Research Centre, Abavas Street 2, Pure, Tukuma district, Latvia

e-mail: Mara.Duma@llu.lv

Abstract

Different vegetables are considered as sources of human health promoting components. Leaf vegetables are widely used in human diet, they are low in calories and fat, but high in dietary fibres, content of minerals, such as iron and calcium and very high in phytochemicals such as vitamin C, carotenoids, lutein and others. On the one hand phytochemicals are a plant’s way of protecting itself. They help shield tender buds and sprouts from predators, the toxic elements, and pollution. On the other hand they have beneficial effect on human health. Genotype along with growing and management conditions can affect the content and the composition of phytochemicals in plants. The aim of research is to determine the content of phytochemicals (chlorophyll a and b, total carotenoids, lutein, vitamin C) in leaf vegetables. The lettuce (Lactuca sativa), spinach (Spinacia oleracea), green and violet basil (Ocinum basilicum), mustard (Brassica juncea) and common nettles young sprouts (Urtica dioica) were grown in greenhouse or in an open field. The content of plant pigments were determined spectrophotometrically, the titrimetric method was used for determination of vitamin C content. It was found out that young nettles sprouts and spinach are the good source of chlorophyll, basil (green and violet) contains more than 0.3 mg 100 g⁻¹ total carotenoids, but for correcting the deficiency of vitamin C it is useful to use mustard, young nettles sprouts as well as leaves of basil.

Keywords: leaf vegetables, phytochemicals.

Introduction

Vegetables play an important role in human diets, as they support the normal functioning of the different body systems. They provide our cells with vitamins, minerals, fibber, essential oils and phytoneutrients. Vegetables contain low amounts of fat and calories (Banerjee et al., 2012).

Leaf vegetables came from very wide variety of plants and they are plants with edible leaves. Each of us knows lettuce and spinach, as well as mustard, but also early springtime nettles are valuable source of vitamin C. Green leafy vegetables are popularly used for food, being a rich source of β-carotene, ascorbic acid, minerals and dietary fiber. One of the most popular vegetable is lettuce. Lettuce is cultivated worldwide, and is one of the most consumed green leafy vegetables in the raw form for its taste and high nutritive value, being regarded as an important source of phytochemicals, including carotenoids, in the diet (Chang et al., 2013).

Nettles are known as widely used as food in early spring. Stinging nettle young leaves added to soups or salads and dried for winter use (Hoijnik et al., 2007). Spinach contains number of different phytochemicals which have a high nutritional value. It is a rich source of antioxidants, especially high in vitamins A, C, E, K, β-carotene, selenium and omega-3 fatty acids as well as rich in lutein and zeaxanthin. Spinach contains microelements such as potassium, calcium, magnesium, manganese, zinc and others. Spinach extracts have several beneficial effects, such as anticancer, antiaging and protecting of central nervous system (Lomnitski et al., 2003).

Sweet basil is a popular herbal crop grown for fresh or dry leaf or essential oils. The leaves of basil are a good source of vitamin P and ascorbic acid, contain microelements zinc and manganese. It have been found, that basil contains high amount of xantophyll (Calucci et al., 2003).

Mustard, like spinach, contains many phyto-nutrients that have health promotional and disease prevention properties. Mustards are very low in calories and fats, but are supposed to be one of the highest among leafy vegetables, which provide vitamin K. It is a rich source of flavonoids, carotenes, lutein and zea-xanthin. Moreover fresh mustard leaves are an excellent source of vitamin C, several essential minerals such as calcium, iron, magnesium, potassium, zinc, selenium, and manganese (Banerjee et al., 2012).

Knowledge on different phytochemical presence and its content in vegetables is important for an appropriate choice of products according to the physiological needs. Phytochemicals are a large group of plant derived compounds, the plant’s way of protecting itself. In addition they appear to have significant physiological effects in the human body. There are more than thousand known phytochemicals. They are acting as antioxidants, stimulating enzymes, interfering with DNA replication, destroying bacteria, as well as they seem to act to reduce the onset of diseases such as cancer and heart diseases (Krishnaswamy, Raghu ramulu, 1998).

The leaves of plants contain number of colour pigments generally falling into two categories chlorophylls and carotenoids. Chlorophylls a and b are the pigments that make plants look green. Chlorophyll is often referred to as the green blood of plants due to the identical molecular structure with hemoglobin with only difference in centre atom (iron or magnesium). This similarity makes chlorophyll so important to our health, it improve digestive, immune and detoxification systems of human body (Kopsell et al., 2005).

Carotenoids are natural pigments that provide the natural yellow, orange or red colours of vegetables and fruits. These colours are a result of the presence of
conjugated double bonds, also providing carotenoids with antioxidant properties. Therefore the interest of carotenoids, which are found in vegetables, is not only due to their provitamin A activity but also to their antioxidant action by scavenging oxygen radicals and reducing oxidative stress in the organism (Rao, Honglei, 2002). They are thought to provide health benefits in decreasing the risk of disease, particularly certain cancers and eye disease. Besides beta-carotene, which is the best-known carotenoid, this group includes alpha-carotene, lycopene, lutein, zeaxanthin and cryptoxanthin. It is known that the largest contribution of vitamin A intake comes from the provitamin A carotenoids in plant food, which may contribute up to 82% of the total vitamin A intake (Van den Berg et al., 2000), therefore the green leafy vegetables are good sources of carotenoids. Carotenoids serve many functions in plants including light harvesting, structure stabilization and excess energy dissipation, they protect plants from free radicals, such as triplet excited chlorophyll (‘Chl) and singlet oxygen (‘O), produced when light intensity exceeds photosynthetic capacity (Mortensen et al., 2001).

Vitamin C (ascorbic acid) is a major antioxidant in the human body. This water-soluble vitamin is involved in many biological processes. Its biological significance is based on the ability to participate in enzymatic and hydroxylation reactions, participates in the oxidation - reduction processes. Vitamin C promotes the absorption of microelements iron and copper, participates in trace element metabolism, and protects cells from damage caused by free radicals, toxins and environmental pollution. It is directly related to protein exchange. Different fruit, vegetables and berries are rich in vitamin C, especially red pepper, black currents and the content of vitamin C may vary depending on the environmental and stress factors such as light intensity, temperature, humidity conditions, air pollution, etc. (Singh et al., 2012).

The aim of study was to determine the content of phytochemicals in leafy vegetables growing and harvested in Latvia.

Materials and Methods

Investigations were carried out at the Latvian University of Agriculture, Institute of Soil and Plant Sciences and Pure Horticultural Research Centre.

Plant material

Six types of leafy vegetables were selected for analysis. There were lettuce (Lactuca sativa) variety ‘Rīga’, spinach (Spinacia oleracea) variety “Matador”, green basil (Ocimum basilicum) variety ‘Genovese’, violet basil (Ocimum basilicum) variety ‘Aromat’, leaf mustard (Brassica juncea) and common nettle (Urtica dioica). Seedlings of lettuce, spinach, basil and mustard were grown in 2 L pots with peat substratum in the polycarbonate greenhouse without artificial illumination. Temperature in green house was maintained between 8 °C (min at night) and 30 °C (max at day time). Vegetation time 2 months. Nettles were grown in an open field. All samples were harvested in one day, when shoots was 10–15 cm long. Samples were cooled, packed and transported to the laboratory ensuring low temperature. In the same day the samples for biochemical analysis were prepared.

Phytochemical extraction and determination

All the reagents used were with the analytical grade from Sigma Aldrich, Germany. UV spectrophotometer UV-1800 (Shimadzu Corporation, Japan) was used for the absorbance measurements.

The chlorophylls, carotenoids and xanthophylls were extracted with ethanol according to the methods described by Kukric et al. (2012) and Chang et al. (2013) with some modifications. For extraction a representative portion of sample (0.1±0.001 g) (mass) was accurately weighted, grinded and quantitatively transmit in a glass test tube. Then ethanol was added till 5 mL to it and the test tubes were held in dark for 15 min with occasional shaking at room temperature and finally centrifuged. The chlorophylls, carotenoids and xanthophylls content were analyzed spectrophotometrically by absorption measurements (A) at 350 to 700 nm with 1 nm interval and calculated according to the following equations:

\[
\text{Chlorophyll a (mg g}^{-1}\text{)} = \frac{13.7A_{665} - 5.76A_{649}}{\text{mass} \cdot 200} \tag{1}
\]

\[
\text{Chlorophyll b (mg g}^{-1}\text{)} = \frac{25.8A_{649} - 7.6A_{665}}{\text{mass} \cdot 200} \tag{2}
\]

\[
\text{Carotenoids (mg g}^{-1}\text{)} = \frac{4.7A_{440} - 0.263 e_{\text{chlorophyl}}}{\text{mass} \cdot 200} \tag{3}
\]

\[
\text{Xanthophylls (lutein) (mg g}^{-1}\text{)} = \frac{11.51A_{480} - 20.61A_{434}}{\text{mass} \cdot 200} \tag{4}
\]

The content of vitamin C was determined titrimetrically using 2,6- dichlorphenolindophenol (AOAC, 1990). For determination ±0.001 g of sample was accurately weighted, grinded in porcelain mortar, than quantitatively transfer in 100 mL tubes, added 50 mL of 1% HCl and 5% HPO₃ mixture (v : v=1 : 1) and mix thoroughly. After 30 minutes solution was filtered through a filter paper No. 89. For determination 10 mL (V_{add}) of filtrate was titrated with 0.0005 molar solution of 2.6 dichlorphenolindophenol (V_{titr}).

The content of vitamin C was calculating according to the equation:

\[
\text{Vitamin C (mg 100 g}^{-1}\text{)} = \frac{V_{\text{add}} \cdot 0.044 \cdot V_{\text{total}}}{V_{\text{anal}} \cdot \text{mass}} \cdot 100 \tag{5}
\]

Experiments were done in 6 replicates and obtained data was elaborated by ANOVA.

Results and Discussion

Genotype along with growing conditions can affect the content and the composition of phytochemicals in
plant. In the present work it was evaluated the content of chlorophylls, total carotenoids, xanthophylls (lutein) and vitamin C in the fresh leaf vegetables grown in Latvia. The content of chlorophylls determined in the analyzed samples is characterized in Table 1.

Table 1  

<table>
<thead>
<tr>
<th>Leafy vegetable</th>
<th>Chlorophyll a, mg g⁻¹</th>
<th>Chlorophyll b, mg g⁻¹</th>
<th>Total chlorophylls, mg g⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lettuce</td>
<td>0.144±0.012</td>
<td>0.044±0.016</td>
<td>0.188±0.015</td>
</tr>
<tr>
<td>Spinach</td>
<td>1.043±0.069</td>
<td>0.461±0.035</td>
<td>1.503±0.102</td>
</tr>
<tr>
<td>Green basil</td>
<td>1.101±0.15</td>
<td>0.330±0.17</td>
<td>1.421±0.05</td>
</tr>
<tr>
<td>Violet basil</td>
<td>1.079±0.098</td>
<td>0.360±0.054</td>
<td>1.439±0.152</td>
</tr>
<tr>
<td>Mustard</td>
<td>0.687±0.105</td>
<td>0.155±0.026</td>
<td>0.824±0.129</td>
</tr>
<tr>
<td>Nettles</td>
<td>1.534±0.26</td>
<td>0.537±0.032</td>
<td>2.070±0.288</td>
</tr>
</tbody>
</table>

Obtained results showed that the richest source of chlorophyll is leaves of young nettle sprouts – the content of total chlorophylls was 10 times higher comparing with lettuce and for 2.5 times higher comparing with leafy mustard. The obtained results confirm the results mentioned in scientific literature (Banerjee et al., 2012, Kukric et al., 2012, Cruz et al., 2012). Hojnik et al. (2007) reported that nettle leaves contain significant amount of chlorophylls till 2.5 mg g⁻¹ fresh weight. Beside other varieties of sweet basil, Kosell et al. (2005) characterized green basil variety Genovese. They reported that content of chlorophyll a is in range from 0.758 till 1.794 mg g⁻¹, chlorophyll b – from 0.149 till 0.344 mg g⁻¹ depending on growing conditions. These results also confirm with our investigations. Taking into account the benefits of chlorophyll it is recommended to expand the young nettle leaves use as food ingredients, especially in springtime, when the intake of biologically active substances is difficult.

The results of research (Fig.1) showed significantly higher content of carotenoids and xanthophylls in young nettle leaves comparing with other analyzed leaf vegetables. The content of carotenoids in leaves of nettles was 0.834±0.072 mg g⁻¹, comparatively in leaves of lettuce the content of carotenoids was only 0.072±0.009 mg g⁻¹. For comparison, Kukric et al. (2012) established that content of carotenoids in leaves of young nettles grown in Serbia ranged from 0.216 to 0.323 mg g⁻¹. The content of carotenoids in analyzed samples of spinach, mustard and basil leaves ranged from 0.255 to 0.398 mg g⁻¹.

Similar tendency was observed analyzing the content of lutein. The leave vegetables which are rich in chlorophyll are also a good source of xanthophylls. In the current research the content of lutein was determined in six leaf vegetables and they can be arranged as follows (starting from vegetable with less content of lutein): lettuce<spinach<mustard<basil violet<basil green<nettle.

Figure 1. Content of total carotenoids and lutein in leaf vegetables

The obtained experimental results are slightly higher than reported in literature. Krumbein et al. (2005) noted that content of lutein in leaf mustard range from 0.034 till 0.089 mg g⁻¹, but Kopsell et al. (2005) reported that lutein content in sweet basil variety Genovese range from 0.04 till 0.07 mg g⁻¹ depending on growing conditions.

Ascorbic acid is considered as one of the most important water soluble vitamins with different important biological functions. Significant amount of vitamin C was determined in mustard and leaves of young nettle sprouts (Fig.2).

Figure 2. Content of ascorbic acid in leaf vegetables

Ascorbic acid content in leaves can be over a wide range depending on cultivar as well as due to the influence of different factors including environmental and growing conditions. Therefore data in literature regarding ascorbic acid content will be very different. The obtained results confirm with investigations of Staugaitis and Viškelis (2001) done in Lithuania as well as Krumbein et al. (2005) reported that leaves of mustard had relatively high content of ascorbic acid till 89.1 mg 100 g⁻¹. In comparison Banerjee et al. (2012) studies found that content of ascorbic acid in
different mustard varieties range between 12.1 till 19.6 mg 100 g⁻¹.
Summarizing the obtained results the conclusion can be drawn that young nettle sprouts and leaves of mustard are very suitable as sources of human health promoting components due to high content of chlorophyll, carotenoids, lutein and ascorbic acid. For example, increased intake of lutein and β-carotene has been associated with decreased risks of cancer and other chronic deceases, especially age-related eyes diseases (Sommerburg et al., 1999), but sufficient amount of vitamin C ensuring a good health due to it antioxidative properties (Martin, 2003). The Regulations No. 988 of Cabinet of Ministers of Latvia, 2009 September 1 determined that the recommended daily intake for adults is 80 mg of vitamin C, but it will achieve 150 mg (Levine et al., 1999). Unfortunately it should be noted that lettuce which is one of the most popular and consumed green leafy vegetables in the raw form especially in spring time contains less phytochemicals comparing with previous mentioned leafy vegetables. Therefore it will be recommended to popularize and increase the consumption of not so traditional leafy vegetables such as leaf mustard and young sprouts of nettles.

Conclusions
The content of total chlorophylls in leaves of young nettle sprouts was 10 times higher comparing with lettuce and for 2.5 times higher comparing with leafy mustard. Basil (green and violet) contains more than 0.3 mg 100 g⁻³ of total carotenoids, but the richest source of carotenoids and lutein is young sprouts of nettles. For correcting the deficiency of vitamin C it is useful to use leaves of mustard, young nettles sprouts and basil.

Acknowledgment
This work was supported by the Latvian Council of Science, project Nr.519/2012

References
THE INFLUENCE OF FERMENTATION TEMPERATURE ON THE DEVELOPMENT OF EXOPOLYSACCHARIDES IN YOGHURT PRODUCTION

Jana Feldmane¹, Inga Ciprovica¹, Paves Semjonovs², Raimonds Linde²

¹ Faculty of Food Technology, Latvia University of Agriculture, 2 Lielā Street, Jelgava, Latvia, e-mail: inga.ciprovica@llu.lv
² Institute of Microbiology and Biotechnology, Latvia University, 4 Kronvalda Boul., Riga, Latvia

Abstract
Exopolysaccharides (EPS) have potential for development and exploitation as functional ingredients with health and economic benefits in dairy industry. The information on the biosynthesis, molecular organisation and fermentation conditions of EPS is rather scarce and the kinetics of EPS formation are poorly described. This study was designed to evaluate the effect of fermentation temperature on the development of lactic acid bacteria (LAB) starters EPS production potential. The commercial starters (Harmony 1.0, TWIST 1.0 and YF-L902, Chr.Hansen, Denmark) are used for yoghurt production. Milk samples were incubated at 38°C, 40°C and 43°C for 7, 6 and 5 hours, respectively, reaching pH 4.5. EPS and lactic acid concentration, LAB colony forming units (CFU) were measured in yoghurt samples using an appropriate analytical technique or standard procedures. The production of intracellularly synthesized EPS varies roughly from 25.28 to 440.81 mg L⁻¹ during fermentation. The fermentation temperature significantly contributes to EPS concentration because the increased rate of fermentation temperature is attributed to increased metabolic activity of LAB. Thermophilic strains produce maximal amounts of EPS under conditions optimal for growth. There isn’t established the correlation between the amount of EPS and CFU of LAB in samples fermention at different temperatures, but there is found stable CFU concentration in samples with higher initial EPS concentration during the shelf life of yoghurt. EPS in their natural environment are thought to play a role in the protection of the microbial cell against desiccation to phagocytosis and phage’s attack, osmotic stress, adhesion to solid surfaces and biofilm formation. The fermentation temperature has a crucial role for the development of yoghurt quality and functional properties.

Keywords: exopolysaccharides, lactic acid bacteria, starters, yoghurt, fermentation.

Introduction
Lactic acid bacteria (LAB) have been extensively studied for their economic importance in food fermentation. LAB secrete exopolysaccharides, which play a significant role in the protection of microbial cell against phagocytosis, phage attacks, antibiotics, toxic compounds, osmotic stress and bacteriocins (De Vuyst, Degeest, 1999; Ruas-Madiedo et al., 2001; O’Connor et al., 2007), and in assuring the proper consistency and texture of fermented dairy products and cheese (Petry et al., 2000; Folkenberg et al., 2006). Additionally, some of EPS have prebiotic properties that could find important applications in functional foods (Ruas-Madiedo et al., 2010; Hidalgo-Cantabrana et al., 2012).

EPS are long-chain polysaccharides consisting of branched, repeating units of sugars or sugar derivatives. These sugar units are mainly glucose, galactose and rhamnose in different ratios. EPS are secreted into cells surroundings during growth and are not attached permanently to the surface of the microbial cell. This distinguishes them from the structurally similar capsular polysaccharides, which do remain permanently attached to the surface of the cell (Cerning, 1990; De Vuyst et al., 2001). The total yield of EPS produced by LAB depends on the composition of the medium and the conditions in which the strains grow, i.e. temperature, pH and incubation time. The production of intracellularly synthesized EPS varies roughly from 0.045 to 0.350 g L⁻¹ when the bacteria are grown under non-optimized culture conditions. Optimal culture conditions result in EPS yields from 0.150 to 0.600 g L⁻¹, depending on the strain (Cerning, 1990; Ruas-Madiedo et al., 2010). Optimal conditions of temperature, pH and incubation time result in improved EPS yields. Several reports show that low temperatures markedly induce slime production of LAB (De Vuyst, Degeest, 1999; Cerning et al., 1992). This effect has been explained, based on information for EPS production from Gram-negative bacteria, by the fact that slowly growing cells exhibit much slower cell wall polymers biosynthesis, making more isoprenoid lipid carrier molecules available for EPS biosynthesis (Petry et al., 2000; De Vuyst et al., 2001). However, several investigators find higher EPS production by LAB strains at higher cultivation temperature (De Vuyst, Degeest, 1999; Degeest et al., 2001; Grobben et al., 1995) and under conditions optimal for growth, for instance, with respect to pH (De Vuyst and Degeest, 1999; Degeest et al., 2001; Mozzı et al., 1994; Mozzı et al., 1996; Degeest, De Vuyst, 1998; Grobben et al., 1998) and oxygen tension (Degeest et al., 2001; Degeest, De Vuyst, 1998; Grobben et al., 1998; Behare et al., 2009). Whereas mesophilic strains seem to produce maximal amounts of EPS under conditions not optimal for growth at low temperatures; EPS production from thermophilic lactic acid bacteria appears to be growth-associated, i.e. maximal production under conditions optimal for growth is observed (De Vuyst et al., 1998). In the case of growth-associated production, EPS biosynthesis generally starts almost simultaneously with growth of LAB, shows a maximum rate when the culture is in its exponential growth phase and reaches a maximum towards the end of the active growth (De Vuyst et al., 1998). It means that during storage time of fermented dairy products EPS biosynthesis would be...
continued with the different speed due to stationary growth phase of LAB.

EPS degradation often takes place upon prolonged incubation (De Vuyst and Degeest, 1999; Mozzi et al., 1996; De Vuyst et al., 1998; Gassem et al., 1997) and further biochemical reactions during the shelf-life of fermented dairy products. This may be due to glycohydrolase activity of LAB (De Vuyst, Degeest, 1999; Cerning et al., 1992; Gancel, Novel, 1994; Cerning et al., 1990). However, a marked reduction in the EPS yield upon prolonged fermentation seems to be dependent on the strain used and both chemical and physical conditions (temperature, pH, etc.) (De Vuyst, Degeest, 1999; De Vuyst et al., 1998; Gancel, Novel, 1994).

This study was designed to evaluate the effect of fermentation temperature on the development of lactic acid bacteria starters EPS production potential.

Materials and Methods

Preparation of samples

Pasteurized and cooled milk samples were inoculated with different starters (given in Table 1) and incubated at 38 °C, 40 °C and 43 °C for 7, 6 and 5 hours, respectively, until pH of coagulum reached 4.5. The amount of starters was added based on the recommendations of starters’ manufacturer Chr. Hansen (Denmark).

The characteristic of commercial starters for yoghurt production used in the study

<table>
<thead>
<tr>
<th>Starter code</th>
<th>Composition</th>
<th>Producer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harmony 1.0</td>
<td>Streptococcus thermophilus, Lactobacillus delbrueckii subsp. bulgaricus, Lactobacillus fermentum</td>
<td>Chr. Hansen, Denmark</td>
</tr>
<tr>
<td>TWIST 1.0</td>
<td>Streptococcus thermophilus, Lactobacillus johnsonii, Lactobacillus delbrueckii subsp. bulgaricus</td>
<td>Chr. Hansen, Denmark</td>
</tr>
<tr>
<td>YF-L902</td>
<td>Streptococcus thermophilus, Lactobacillus delbrueckii subsp. bulgaricus</td>
<td>Chr. Hansen, Denmark</td>
</tr>
</tbody>
</table>

Fermented milk samples were stirred and maturated at 4–6 °C for 10-12 hours and stored at 4–6 °C for 7 days.

Determination of pH, CFU of LAB and EPS

An amount of EPS synthesized by starters’ cultures, pH and colony forming units (CFU) of lactic acid bacteria were detected in yoghurt samples after maturation and at the end of the shelf-life of yoghurts. MRS medium (de Man Rogosa & Sharpe; Scharlau, Spain) was used for analysing CFU of LAB. pH of samples was measured by pH-meter Jenway 3520.

EPS were determined in yoghurt samples according to below given methodology (Ruas-Madiedo, de los Reyes-Gavilán, 2005).

Isolation of EPS

300–500 mL of fermented sample were put into the laboratory flask and boiled in water bath at 100 °C for 30 minutes. After cooling samples were centrifuged at 8000 min⁻¹ for 10 minutes and 17 mL of 85% trichloracetic acid were added to 100 mL of sample. Samples were cooled up to 4 °C and again centrifuged at 8000 rpm for 10 minutes. Precipitation of EPS from samples was prepared using cold ethanol (-20 °C, 1:3). Samples were stood in the fridge for 48 h and late centrifuged (4 °C, 8000 min⁻², for 10 minutes), dissolved precipitation in dH₂O and defined EPS.

Quantification of EPS

5% phenol solution in water (dissolve 5 g fresh phenol in dH₂O and fill up to 100 mL into the flask) was prepared. Also 1 mg mL⁻¹ glucose solution (dissolve 250 mg glucose in dH₂O and fill up to 250 mL into the flask) was prepared. For obtaining of calibration line, glucose solutions prepared in different proportions in 6 eppendorfs were used. 400 µL of sample was put into a glass tube and added 400 µL of 5% phenol solution in water. For controlling, 400 µL dH₂O + 400 µL of 5% phenol solution in water was used.

After that, 2 mL of concentrated sulphuric acid were added sharply into the solution in tube. Let the samples stood for 10 minutes, then stirred and let them stood for 10 minutes at 30 °C. Samples at 490 nm in quartz cuvettes were measured and compared with the control sample. The amount of EPS (mg) was calculated using glucose calibration line.

Statistical analysis

Descriptive statistics was carried out to determine the differences of produced EPS concentration in different samples by Microsoft Windows for SPSS 14.0 software. Correlation analysis was used for determination of the differences between EPS concentration and CFU of LAB in yoghurt samples.

Results and Discussion

Exopolysaccharides production of LAB is an important attribute for the fermented dairy products (Jolly et al., 2002; Welman, Maddox, 2003; Ruas-Madiedo et al., 2010). Many of the thermophilic microorganisms produce exopolysaccharides and are of great technological importance. Thermophilic lactic acid bacteria starters show an optimum temperature ranging around 40–43 °C. By providing the microorganisms in their optimum temperature range, incubation time of approximately 5 hours was achieved. It was generally accepted that the lower fermentation temperature the longer it takes to reach a certain pH and the firmness of the final products. Milk acidification was carried out for all yoghurts until pH 4.5. Then, they were rapidly cooled and stirred before storage at 4–6 °C. For better understanding the influence of different fermentation temperatures on the functional properties of yoghurt samples, especially EPS concentration and colony...
forming units of LAB, pH dynamics of samples during fermentation was studied.

pH dynamics is quite similar during fermentation of samples in the same temperature. At the end of fermentation the pH of yoghurt samples ranged from 4.50 to 4.57 in the analysed samples using different starters and fermentation temperatures. The residual acidification activity was observed for all samples during the shelf-life with stable pH values ranging from 4.25 to 4.28 (Harmony 1.0), from 4.16 to 4.20 (YF-L902) and from 4.30 to 4.35 (TWIST 1.0) depending on conditions for fermentation. However no significant effect of EPS concentration was observed on yoghurt postacidification during the shelf-life and according to the findings of Doleyres and co-authors (2005) no significant effect of EPS was observed on yoghurt postacidification.

The production of intracellularly synthesized EPS in yoghurt samples varies roughly from 25.28 to 440.81 mg L$^{-1}$ during fermentation.

Table 2

<table>
<thead>
<tr>
<th>Starter</th>
<th>Fermentation temperature, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>38 °C</td>
</tr>
<tr>
<td>Harmony 1.0</td>
<td>32.10±6.33</td>
</tr>
<tr>
<td>YF-L902</td>
<td>137.07±8.61</td>
</tr>
<tr>
<td>TWIST 1.0</td>
<td>228.78±6.33</td>
</tr>
</tbody>
</table>

The fermentation temperature significantly contributes to EPS concentration because the increased rate of fermentation temperature is attributed to increased metabolic activity of LAB (Figure 1, a, b, c).

Our results are comparable with the findings of several authors (De Vuyst, Degeest, 1999; Laws et al., 2001; De Vuyst et al., 2001) that the higher EPS production of LAB was observed at the higher cultivation temperature, because of this grown-associated EPS production with thermophilic starter cultures and because of the limited number of catabolic pathways that provide energy in LAB (substrate level phosphorylation and secondary metabolic energy generation), cell synthesis is limited, and so is the energy-demanding EPS biosynthesis.

We also observed EPS degradation during the self-life of yoghurt. The reduction of EPS yield seems to be dependent on the starter used and biochemical reactions due to LAB metabolism during the storage of samples. The highest degradation was observed in yoghurt samples where after fermentation and maturation were determined the higher yield of EPS (in samples fermented with starters TWIST 1.0 and YF-L902 at 40 °C and 43 °C).

Figure 1. The comparison of EPS concentration in yoghurt samples after fermentation and at the end of shelf-life

We also noticed the tendency to increase EPS concentration during the self-life of product (in samples fermented with starters Harmony 1.0 at 38 °C, 40 °C and 43 °C; TWIST 1.0 at 38 °C and 40 °C; YF-L902 at 38 °C). A possible interpretation of our results in thermophilic starters is that isoprenoid phosphate carriers are primarily needed for cell wall synthesis growth (Petry et al., 2000). This may explain our finding that EPS production is increased in stationary phase cells.

EPS production influences the amount of CFU of LAB in yoghurt samples (Figure 2, a, b, c).

There isn’t established the correlation between the amount of EPS and CFU of LAB in samples fermented at different temperatures, but there is found stable CFU concentration in samples with higher EPS.
concentration during the shelf-life of yoghurt (Figure 2, b and c). We could explain it with EPS functional properties that EPS in their natural environment are thought to play a role in the protection of the microbial cell.

![Graph showing changes of CFU of LAB in yoghurt samples after fermentation and during the shelf-life](image)

**Figure 2. The changes of CFU of LAB in yoghurt samples after fermentation and during the shelf-life**

We also observed that yoghurt samples with the highest initial EPS concentration and the highest degradation level of EPS showed stable CFU of LAB during the shelf-life of samples. We found that the higher reduction of CFU of LAB was noted in yoghurt samples with the increasing yield of EPS during the shelf-life (Harmony 1.0 at 43 °C, YF-L902 at 38 °C). We explain it with the energy-demanding EPS biosynthesis instead of the growing activity of LAB.

A lot of work has been done in the field of isolating and characterising the composition of EPS produced by various strains of lactic acid bacteria (Faber et al., 2002; Grobben et al., 2000; Petry et al., 2000; Van Calsteren et al., 2002; Ruas-Madiedo et al., 2005) but the functionality of EPS in fermented milk is still not completely clear. The fermentation temperature has a crucial role for the development of yoghurt quality and functional properties, too.

**Conclusions**

Analysing starters’ producing EPS showed large variations in concentration from 25.28 to 440.81 mg L⁻¹ in samples fermented in different temperatures. The fermentation temperature significantly contributes to EPS concentration because the increased rate of fermentation temperature is attributed to increased metabolic activity of LAB.

The correlation between the amount of EPS and CFU of LAB in samples fermented at different temperatures is not established, but there is found stable CFU concentration in samples with higher EPS concentration during the shelf-life of yoghurt.

**References**

aqueous solution of the exopolysaccharide produced by *L. helveticus* 766. *Biopolymers*, No. 63, p. 66–76


EVALUATION OF PRESERVATION CONDITIONS ON NUTS PROPERTIES

Raquel P. F. Guiné1,2, Cátia F. F. Almeida2, Paula M. R. Correia2,3

1 CI&DETs / Polytechnic Institute of Viseu, Campus Politécnico, Repeses, Viseu, Portugal, e-mail: raquelguine@esav.ipv.pt
2 Department of Food Industry, ESAV, Quinta da Alagoa, Ranhados, Viseu, Portugal
3 CI&DETs / Polytechnic Institute of Viseu, Campus Politécnico, Repeses, Viseu, Portugal

Abstract

Dried fruits like nuts are very much appreciated, but because of their hygroscopicity may be susceptible to undesirable changes during storage. Therefore, this work was undertaken to study the effect of different storage conditions on three types of nuts commonly consumed in Portugal (almond, hazelnut and walnut). The samples were originated from different countries and while most had the internal skin on, one sample had it off. The storage conditions tested were: ambient temperature, high temperatures (30 and 50 °C) and low temperatures (refrigeration at + 2 °C and freezing at – 15 °C). The characteristics evaluated were water activity, moisture content, colour and texture.

The results obtained in the present work allowed concluding that the storage conditions that best preserve the characteristics of nuts are those at low temperatures, because, while the treatments at high temperatures induced in general more changes, the refrigeration and freezing systems had a lower effect on the products characteristics, particularly moisture, water activity, hardness and friability. Also the results indicate that the nuts stored under all conditions tested had values of water activity lower than 0.6, thus guaranteeing stability at the microbial and enzymatic levels. It was further concluded that the internal skin had a great influence on the characteristics of the nuts, particularly texture and colour, for all treatments tested.

Keywords: almond, colour, hazelnut, walnut, texture.

Introduction

Tree types of nuts are used worldwide in confectionary, culinary and bakery food applications due to their desirable flavour attributes and high energy density. Almonds (Prunus dulcis, syn Prunus amygdalus, Amygdalus communis L. and Amygdalus dulcis Mill) are one of the most important commercial tree nut crops worldwide (Xiao et al., 2014). Almonds provide important nutrients such as vitamin E, monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), arginine, and magnesium. Almonds also contain considerable amounts of potential prebiotic indigestible carbohydrates (Bolling et al., 2010; Liu et al.).

The common hazel plant (Corylus avellana L.) is a shrub native to Europe and Asia that grows in temperate climates. Hazelnut plays a major role in human nutrition and health because of its special composition of fat (around 60%), most of which are highly rich in MUFA (mainly oleic), protein, carbohydrate, dietary fiber, vitamins (vitamin E), minerals, phytosterols (mainly β-sitosterol), squalene and antioxidant phenols (Oliveira et al., 2008; Yada et al., 2013).

Walnut tree (Juglans regia L.) was originated from Southeast Asia and east Mediterranean areas and was introduced in Europe by the Romans. Walnuts, despite the pleasant taste, were considered for long, inconvenient for human consumption because they are very rich in fat. However they bring a great benefit to health because they help control bad cholesterol and therefore help to protect the heart. They are particularly rich in minerals like copper and zinc, required for formation of haemoglobin in the blood (Almeida, 2013; Tapia et al., 2013). The storage conditions are of major importance so as to maintain the integrity and quality of nuts, and thus prevent spoilage (Freitas-Silva, Venâncio, 2011; Ma et al., 2013). Post-harvest handling and storage might deteriorate both nut nutritional value and taste. It is well known that many factors, such as a high drying temperature or prolonged storage under air and / or at a relatively high temperature and humidity have negative effects on various nut quality attributes (Tsantili et al., 2011).

The aim of this work was to study the effect of different storage conditions on nuts (almond, hazelnut and walnut), namely ambient temperature, high temperatures and low temperatures. The characteristics evaluated were water activity, moisture content, colour and texture.

Materials and Methods

Sampling

The fruits evaluated in this study were almond, hazelnut and nut. The fruits were all without the outer shell, but in some cases they had the inner skin (seed coat) and in other cases not.

The almond samples were: from Spain with skin (A-SP-s), Portugal with skin (A-PT-s) and from United States with and without skin (A-US-s and A-US-n). The hazelnut samples were: from Spain with skin (H-SP-s), Portugal with skin (H-PT-s) and from Turkey without skin (H-TR-n). The walnut samples were: from Chile with skin (W-CH-s), Portugal with skin (W-PT-s), Romania with skin (W-RO-s) and USA with skin (W-US-s).

Determination of water activity and moisture content

The water activity was measured at 25 °C by a hygrometer (Rotronic Hygroskop BT-RS1) connected to a thermal bath. In all cases four determinations were made to calculate the medium value and standard deviation. Moisture content was evaluated by drying in
a stove (WTB-Binder) at 105 °C until reaching constant weight. In all cases three determinations were made.

**Evaluation of colour**

The colour of all samples was measured using a handheld tristimulus colorimeter (Chroma Meter-CR-400). The parameters measured were the lightness L*, which varies between 0 and 100 (from black to white, respectively), and the coordinates of opposed colour: a* and b*, which vary from −60 to +60, where the a* assumes negative values for green and positive values for red, while b* assumes negative values for blue and positive for yellow. The total colour change (ΔE), was the parameter considered for the overall colour difference evaluation, between a sample and the reference fruit (without storage, designated with an index 0):

\[
\Delta E = \sqrt{(L_0 - L^*)^2 + (a_0 - a^*)^2 + (b_0 - b^*)^2}
\]  

**Evaluation of texture**

The texture profile analysis (TPA) for all samples was made by a texturometer (TA.XT.Plus from Stable Micro Systems). The text performed was measure force in compression and the probe used was a Blade Set HDP/BS (Warner-Bratzler). Twenty measurements were carried for each fruit and each storage condition. The curve force (N) versus distance (mm) allows to calculate the hardness (the force at first peak) and friability (the distance of first peak).

**Storage conditions**

The fruits were stored without package for 90 days at ambient conditions, in a stove at two temperatures with controlled temperature but no control over relative humidity, under refrigeration and frozen (Table 1). The use of high temperatures (50 °C) aimed at verifying the effect of these storage conditions on the water absorption and therefore the conservation.

<table>
<thead>
<tr>
<th>Storage conditions for the fruits studied</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Description</strong></td>
</tr>
<tr>
<td>B: Just acquired (base)</td>
</tr>
<tr>
<td>AT: Ambient temperature</td>
</tr>
<tr>
<td>S30: Stove (T controlled but RH not)</td>
</tr>
<tr>
<td>S50: Stove (T controlled but RH not)</td>
</tr>
<tr>
<td>R: Refrigerated</td>
</tr>
<tr>
<td>F: Frozen</td>
</tr>
</tbody>
</table>

**Results and Discussion**

Figure 1 shows that the moisture content of the almond samples was always higher before storage, regardless of the conditions. Comparing the different origins, the almond from Spain had the highest moisture content.

**Figure 1. Moisture content of nuts**

AT – Ambient temperature; S30, S50 – storage at high temperature (30 and 50 °C); R – refrigerated; F – frozen; Origin: SP – Spain, PT – Portugal, US – United States, RO – Romania, CH – Chile, TR – Turkey

As to the almonds from the United States, the absence of the skin contributed to a further dehydration, because the moisture content of the almond with skin was 1.74% while for the sample without skin was 1.46%. The results also reveal that for all almonds the frozen samples were those that had the moisture content more similar to the base ones. The trend observed for almonds was also observed for hazelnuts and walnuts. However, the relative contents are different, where almonds have higher moisture contents when compared to the other two nuts studied. The values of the water activity for all samples and all storage conditions are presented in Figure 2. The results show that, like what was observed for moisture, the storage of 50 °C produces the almonds with lower
water activity, while the frozen almonds showed the higher water activity among the stored samples. The results for the other two nuts studied, hazelnuts and walnuts, are very similar, but once again the magnitude of the values is different, so that the almonds present much higher water activity. One important issue concerning stability and that may influence is lipid quality oxidation, but that was not studied in this work.

However, and despite the slight changes observed, these findings allow to conclude that in general the samples are stable and not susceptible to spoilage, since for all storage conditions the values of aw are lower than 0.6, thus preventing the development of any bacteria, fungi or yeasts.

Table 2 shows the values of the colour parameters for all the nut the samples before storage. The values of \( L^* \), \( a^* \) and \( b^* \) are very similar for the three almond with the skin, but differ much of that of the sample without skin, as naturally expected. The almond samples with the skin present values of \( L^* \) (brightness) under 50, thus indicating that they are very dark, contrasting with the value of the sample without skin (\( L^*=78.1 \)), which stand in the region approaching white. The values of \( a^* \) are positive in all cases, thus indicating that the samples have a more or less (depending on the presence of the skin) reddish colouration. In the case of the A-US-n sample, the value is very close to zero, indicating that the predominance of red is very weak. Regarding the values of \( b^* \), they are all strongly positive, and so the intensity of the yellow colour is high.

As to the colour parameters for hazelnut, the results are very similar to those of the almonds and the trends observed are the same as discussed above. Also the influence of the internal skin is well evidenced.

Regarding the walnut samples, and because they were in this case all with the internal skin, the results are very close among each other. However, it is worth mentioning that the values of \( a^* \) and \( b^* \) are smaller in the skin of the walnuts as compared to the skin of the other two nuts studied.

Figure 2. Water activity of nuts

<table>
<thead>
<tr>
<th>Sample</th>
<th>( L^* )</th>
<th>( a^* )</th>
<th>( b^* )</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-SP-s</td>
<td>47.8±3.3</td>
<td>16.5±1.7</td>
<td>32.7±3.3</td>
</tr>
<tr>
<td>A-PT-s</td>
<td>43.4±2.9</td>
<td>16.8±1.5</td>
<td>30.5±2.7</td>
</tr>
<tr>
<td>A-US-s</td>
<td>47.3±2.6</td>
<td>17.2±1.1</td>
<td>33.9±2.2</td>
</tr>
<tr>
<td>A-US-n</td>
<td>78.1±2.2</td>
<td>16.6±1.2</td>
<td>28.1±2.1</td>
</tr>
<tr>
<td>H-SP-s</td>
<td>42.6±4.1</td>
<td>17.8±1.9</td>
<td>24.5±3.3</td>
</tr>
<tr>
<td>H-PT-s</td>
<td>47.2±4.4</td>
<td>18.6±2.0</td>
<td>26.2±2.6</td>
</tr>
<tr>
<td>H-TR-n</td>
<td>75.2±3.1</td>
<td>4.8±1.5</td>
<td>31.6±3.1</td>
</tr>
<tr>
<td>W-CH-s</td>
<td>40.6±6.4</td>
<td>11.5±1.9</td>
<td>24.2±2.4</td>
</tr>
<tr>
<td>W-PT-s</td>
<td>48.2±6.3</td>
<td>9.4±2.4</td>
<td>28.4±3.7</td>
</tr>
<tr>
<td>W-RO-s</td>
<td>49.3±6.0</td>
<td>10.3±1.8</td>
<td>31.1±2.8</td>
</tr>
<tr>
<td>W-US-s</td>
<td>47.0±6.4</td>
<td>8.9±1.9</td>
<td>26.7±4.1</td>
</tr>
</tbody>
</table>

Figure 3 shows the total colour difference between each sample and the reference, which consisted of the nuts before storage. The values were calculated by equation (1) from the mean values for each case, and therefore no standard deviation is included. The results show that the highest differences were observed for those almonds stored at 50 °C, and that is particularly visible for the almonds with skin. These results indicate that the skin is affected by heat, becoming darker due to the browning processes occurring during storage.
Hence, the results seem to indicate that maybe refrigeration and freezing could be an alternative to preserve the colour of the almonds with skin.

In relation to the other two nuts, it was also observed that the storage at 50 °C originated the highest colour differences. As to the best method of storage for these two nuts, although in majority of the cases it is freezing like in the case of almonds, there are however some exceptions, in the case of hazelnuts from Portugal and from Turkey.

Hardness is the mechanical strength to crush. It is important as it ensures the physical integrity of the product, allowing it to support the mechanical stress in the process of packing and transportation. The hardness limits are specified according to the diameter and weight of the sample and refers to the minimum resistance to be removed from the container without breaking. The textural attribute hardness was evaluated for almonds and hazelnuts and the results are shown in Figure 4.

The almonds from different origins show important differences in terms of this characteristic, being the almonds from Spain the softer among the samples with skin. Naturally that by removing the skin, the hardness decreases, as the results of the two sample from US (with and without skin) indicate. As to the effect of storage conditions, the results are not consistent among the different almonds, because the same treatment produces contradictory effects on different almonds. However, in general, the refrigeration and freezing conditions allow obtaining values closer to the reference samples of almonds.

As to the hazelnuts, it can be observed from Figure 4 that the variability is lower when compared to the almonds, and so are also the values of hardness. In fact, the hardness of the almond samples is almost double of the hazelnut samples. In the case of hazelnuts, all the storage conditions allow obtaining products with a similar hardness to the unstored samples.

The friability respects to the ease with which the fracture occurs in the products, and assumes a particular importance to avoid product loss. Figure 5 presents the results obtained for friability of almonds and hazelnuts, and these are quite consistent with what was previously observed for hardness. Once again the almond without skin shows to be less affected by storage, while the almond from Spain in that which suffers higher changes along storage, regardless of the conditions. Figure 5 also reveals that hazelnuts have lower friability when compared to almonds.
Conclusions

The findings of the present work allowed to draw some general conclusions as to the best storage conditions to preserve the characteristics of nuts. In fact, while the treatments at high temperatures induce in general more changes, the refrigeration and freezing systems are recommended for their lower effect on the products characteristics.

Furthermore, it was also concluded that the nuts stored under all conditions tested were safe from the point of view of the microbiological stability, given the very low water activity observed in all cases. Finally, it is important to note that the presence or absence of the internal skin greatly influences the product properties.

Acknowledgment

The authors thank the Portuguese Foundation for Science and Technology (FCT) and Research Centre CI&DETS for financial support (project PEst-OE/CED/UI4016/2014).

References

THE USE OF FACE READING TECHNOLOGY TO PREDICT CONSUMER ACCEPTANCE OF CONFECTIONERY PRODUCTS

Grazina Juodeikiene¹, Loreta Basinskienė¹, Daiva Vidmantienė¹, Dovile Klupsaitė¹, Elena Bartkiene²

¹ Department of Food Science and Technology, Faculty of Chemical Technology, Kaunas University of Technology, Radvilenu Rd. 19, Kaunas, Lithuania, e-mail: loreta.basinskienė@ktu.lt
² Department of Food Safety and Quality, Faculty of Veterinary Academy, Lithuanian University of Health Sciences, Tūžes str. 18, Kaunas, Lithuania

Abstract

Traditional sensory and consumer tests predict consumer acceptance of new products rather poorly, as evidenced by the high their failure rates in the market. These tests typical reflect conscious processes whereas consumer acceptance may also be based on unconscious processes.

The aims of this work were to examine whether facial expressions measured with the Noldus FaceReader technology can be used for differentiating between differing sugar confectionery products (various types of sweets and chocolates), and to investigate whether facial reactions are able to explain liking ratings on hedonic scales.

Naive consumers (mean age 22 years) were recruited at the Kaunas University of Technology. They were asked to rate the sample with an intentional facial expression, which was recorded and then characterized by FaceReader program (Noldus Information Technology, Wageningen, The Netherlands). The measurements showed significant differences between facial expressions elicited by the different samples of tested sugar confectionery products and reflected the introspective liking ratings well. The positive correlations of facial expression “happy”, and negative correlations of “sad” expression intensity against self-reported liking ratings suggest that these may be the most valuable descriptors for explaining the self-reported hedonic quality of sweets and chocolates. It can be concluded that Noldus FaceReader technology is sufficiently accurate for differentiating between sugar confectionery products and can deliver additional information to conventional acceptance tests.

Keywords: sugar confectionery products, consumers, facial expressions, hedonic liking, FaceReader.

Introduction

Up to 80% of all new food products fail in the marketplace, despite the fact that they are typically subjected to a large number of sensory and consumer tests before their market introduction. This suggests that the standard sensory and consumer tests, which typically include sensory analytical profiling and liking tests, have a low predictive validity with respect to general product performance. Possibly, consumer food choice outside the laboratory may be less based on cognitive information processing and rational reasoning, and more on unarticulated / unconscious motives and associations (Dijksterhuis, Smith, 2005; Köster, 2009). Reasons for likes or dislikes of different foods are typically difficult to articulate but may determine much of our food choice. Certain foods are more attractive than other foods because for some reason they make us feel good – i.e. they trigger positive emotions.

For some time, sensory analysts within the commercial sector have looked for better means to connect with marketing. The measurement of emotions might help in the further connection of sensory science and marketing. The measurement of emotions also serves as a further tool to support product development. Measurement of emotions allows us to compare existing products, and measure the emotional response to product prototypes. In these ways, the measurement of emotions can provide a common lexicon for sensory and marketing to communicate and for product development that meet a marketing need. Emotions can be the common language to bring these areas together.

There are many studies showing that tastes and odours elicit different emotions and facial reactions in neonates (Soussignan et al., 1997) children (Soussignan, 1996; Zeinstra et al., 2009) and adults (Greimel et al., 2006; Wendin et al., 2011). In most of these studies quite intense stimuli were used, like for example, concentrated basic taste solutions (Wendin et al., 2011) or odours ranging from fruity to fecal (Soussignan, 1996). The study of emotions in relation to food choice has recently advanced by work of King and Meiselman (2010) and De Smet and Schifferstein (2008). Their results suggest that most emotions related to food are mildly positive, are only partly related to liking, and improve the predictions of food choice.

Facial expressions can be analyzed with the anatomically based Facial Action Coding System (FACS). These FACS analyses are very time-consuming and require trained observers. To overcome these difficulties, different automated facial expression recognition systems like Nviso (Nviso SA, Lausanne, Switzerland), Affdex (Affectiva Inc., Waltham, USA) and FaceReader (Noldus Information Technology, Wageningen, The Netherlands) have been developed. These systems are capable of analyzing facial expression patterns from video data. Currently, these systems are used mainly for research in the fields of psychology, education, market research and consumer behaviour. De Wijk et al. (2012) analyzed the facial expressions elicited by the prospect of tasting or smelling liked or disliked food with FaceReader. The first sight of disliked foods compared to liked foods resulted in increased facial expressions of sadness, disgust, and anger. However up until now, little work has been published about the measurement of facial expressions elicited directly by the actual tasting of food products using facial expression recognition.
technology. Danner et al. (2014) examined consumers’ facial reactions elicited by the flavour of orange juice products using FaceReader technology in implicit and an explicit measurement approach. Both, implicit and explicit measurements showed significant differences between facial expressions elicited by the different samples. The explicit measurement reflected the introspective liking ratings well. Especially expressions happy and disgusted showed a high correlation with liking and were good indicators for liked and disliked samples, respectively. To minimize artefacts, caused by eating and drinking, which can be easily misinterpreted by the FaceReader software as emotion, they used liquid samples (juice) which need less processing in the mouth than solid samples.

The aim of this work was to examine whether facial expressions measured with the Noldus FaceReader technology are a sufficiently accurate measure for differentiating between various types of sugar confectionery products (sweets and chocolates), and to investigate whether facial reactions are able to explain such product liking ratings on hedonic scales.

Materials and Methods

Samples and sample preparation

Six different types of sugar confectionery products were tested in the experiment. Each set of tested products was made from 3 to 4 samples with significant differences in flavour or texture (Table 1). All samples were presented in a sequential way, at room temperature (20–22 °C), randomized and coded. Water was provided to rinse the mouth before and between tasting the samples. All tested sugar confectionery products were commercial products delivered by various Lithuanian companies.

Table 1

<table>
<thead>
<tr>
<th>Samples of sugar confectionery products</th>
<th>Products</th>
<th>Significant difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chocolate bars</td>
<td>Different additives: peanuts, hazelnuts, almonds, coffee</td>
<td></td>
</tr>
<tr>
<td>Milk chocolate</td>
<td>Different brand names: Milka, Sonata, Pergale</td>
<td></td>
</tr>
<tr>
<td>Sweets “Ruta”</td>
<td>Different fillings: almond, hazelnuts, actinidia, granadilla and white chocolate</td>
<td></td>
</tr>
<tr>
<td>Sweets “Ruta” with nutty filling</td>
<td>Different coatings: dark (70% or 50% cocoa solids), white, milk chocolate</td>
<td></td>
</tr>
<tr>
<td>Sweets “Ruta” with almond filling</td>
<td>Different coatings: dark (70% or 50% cocoa solids), white, milk chocolate</td>
<td></td>
</tr>
<tr>
<td>Caramel “Tropic”</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Participants and measurements

The number of participants in the testing of each type of sugar confectionery products varied from 12 to 20. All participants were students of the Department of Food Technology, Kaunas University of Technology, with an average age of 22 years.

The experiments took place in the Sensory Laboratory at Kaunas University of Technology. At first, participants were introduced to the procedure for tasting. They were asked to taste the whole presented food sample (10 g) at once, take some seconds to think about an impression of it, then give a signal with their right hand and visualize the taste experience of the sample with a facial expression best representing their liking of the sample (explicit measurement). Afterwards, they rated their liking or disliking of the sample on a 7-point hedonic scale, ranging from 1 (dislike extremely) to 7 (like extremely).

The whole procedure was filmed with a Microsoft LifeCam Studio webcam, mounted on the laptop facing the participants, using Media Recorder software (Noldus Information Technology, Wageningen, The Netherlands). The recordings with a resolution of 640×480 at 25 frames per second were saved as AVI files and analyzed frame by frame with FaceReader 5 software (Noldus Information Technology, Wageningen, The Netherlands), scaling the 6 basic emotion patterns (angry, happy, disgusted, sad, scared, surprised) and neutral from 0 (not present at all) to 1 (maximum intensity of the fitted model). For each sample, the section of intentional facial expression (exactly from the point when the subject had finished raising their hand to give the signal until the subject started lowering the hand again) was extracted and used for the statistical analysis. FaceReader contains an image quality bar, which gives a good indication of how well the program is able to model the face depicted in the image. For the best image quality, the main attention was focused on camera position and illumination. For this reason, participants were asked to sit and look frontally into the camera. All of the participants agreed to the use of their data in the context of this experiment.

Statistical analysis

For the statistical analysis, the maximum values of the facial expression patterns (angry, disgusted, happy, sad, scared, surprised, and neutral) of the respective section were used. To examine the correlation between facial expressions and the hedonic liking, a Multiple Linear Regression was performed. All analyses were performed with STATISTICA V10 (StatSoft, Inc., Tulsa, OK, USA). Significance of differences between treated samples was evaluated by using Duncan’s multiple range tests at a 5 percent level (p<0.05).

Results and Discussion

Results of experiments showed that there were significant differences in facial expressions between tasted types of sugar confectionery product (Fig. 1) as well as between the samples of the same type of products (Fig. 2). Tested types of confectionery products differed significantly concerning the emotion patterns happy, sad and angry. The similar tendencies were found during the analysis of the same type of
confectionery products (sweets). The sweets also were differ in emotion pattern surprised. No significant differences (p>0.05) in other facial expressions between tested samples were observed.

It was noticed, that the caramel samples elicited significantly more intense facial reactions of happy than all other tested confectionery products. Sweets “Ruta”, especially with dark chocolate (70 % cocoa) elicited the strongest sad facial expressions, significantly differing from all others. The statistical analyses of the self-reported liking ratings identified the caramel “Tropic” as the most liked sample and the chocolate bars as the least liked. The data collected suggest that emotional intensity sometimes tracks with acceptance, and sometimes differs. For example, the acceptances of sweet with different fillings do not track with the emotion profile (Fig. 2). Thus emotions might help to explain acceptance data and why acceptance data might not always predict market success.

While the measurement of emotions gives new information beyond acceptance, it is nevertheless interesting to relate emotions and acceptance. Linear regressions of the mean values of the facial expressions showed positive correlations of happy and negative correlations of sad (Table 2). This indicates that liked samples elicited more intense facial expressions of happy than disliked samples.

This study shows that measuring facial expressions using Noldus FaceReader 5 is a sufficiently accurate method to differentiate between various sugar confectionery samples. Discrimination between liked and disliked samples was possible on the basis of the intensity of elicited happy and sad facial expressions. This supports the findings of Danner et al. (2014) who used the FaceReader technology for study of orange juice and found high correlation of facial reactions happy and disgusted with liking.

It should also be mentioned that some differences in the intensity of facial reactions between participants were observed. The participants could be divided into two groups, the ones who showed clearly visible facial reactions when tasting the samples and those who had a poker face showing only little to almost no facial reactions. This can partially be attributed to the sensory laboratory test setup, where the participants are facing an unfamiliar environment and may feel stressed to a certain degree, or are very concentrated on the task.

Further examinations in a more natural environment, also if possible without directly asking the participants to rate the products, could be interesting.

It is also important to point out the limitations and requirements of FaceReader technology. It does not work with children below the age of three. Pose, movement and rotation of the test person are limited. The test person needs to face the camera head on (angle <40°). The face must not be partially obscured by hair or when handling samples.

Motor artefacts, caused by eating and drinking, can be misinterpreted by the FaceReader software as emotion. In more complex tasting situations, like full meals that involve longer and potentially overlapping oral processing actions, motor artefacts can compromise the measurement of facial expressions to a higher degree.

**Conclusions**

It could be concluded that NoldusFaceReader technology is sufficiently accurate to detect significant differences in facial expressions elicited by different
samples of sugar confectionery products, such as chocolates, sweets, caramel, and can deliver additional information to conventional acceptance tests. However, more research is needed to see how this technology performs in more complex testing procedures, simulated or real life environments. The positive correlations of happy and negative correlations of sad expression intensity against self-reported liking ratings suggest that these may be the most valuable descriptors for explaining the self-reported hedonic quality of sugar confectionery products.

Acknowledgment

The research was funded by a grant (EUREKA ITEA 2 project 11005 Empathic) from Lithuanian Agency for Science, Innovation and Technology.

References

THE WETTABILITY AND SURFACE FREE ENERGY OF ACORN STARCH GELS ISOLATED BY ALKALINE AND ENZYMATIC METHODS
Paula M. R. Correia1,2, Susete Gonçalves1, Helena Gil1, Jorge Coelho3
1 CI&DETs / Polytechnic Institute of Viseu, Campus Politécnico, Repeses, Viseu, Portugal, e-mail: paularcorreia@hotmail.com
2 Department Food Industry, ESAV, Quinta da Alagoa, Ranhados, Viseu, Portugal
3 Faculty of Science and Technology of Coimbra University, Rua Silvio Lima Univ. Coimbra - Pólo II, Coimbra, Portugal

Abstract
Wettability may be a convenient parameter providing information on surface properties of starch gels surface. In food technology, a better understanding of the surface properties of gels can be useful in developing new food products and food components. The gelatinized starch has the property of forming gels, and when it is dehydrated, it forms a hard, transparent, bright, and resistant film. Starch biofilms are produced from gelatinization and after retrogradation of starch. This work aimed to study acorn starch gels wettability and their surface free energy, as well as the colour. Quercus suber and Quercus rotundifolia fruits were dry and flour were produced. Starch was isolated from acorn flours by an alkaline and enzymatic laboratory scale methods. Starch gels films were prepared with starch suspensions of 1.2 and 3% from the isolated acorn starches. Contact angles were measured on dry starch gel films. Colour of films was measured using a colorimeter and the classification by CIELAB system. The isolation method influenced the wettability of the acorn starch films form heated 3% starch suspensions. The acorn films presented high contact angles, meaning that films are hydrophobic, with less water affinity, for both starch isolation method, between 69.9–74.3° for Q. rotundifolia and 68.6–70.2° for Q. suber. The surface energy was different for acorn species and isolation methods, and generally it is low, which means that acorn starch presented high intramolecular interactions forces. Films also presented similar transparency and were yellow-brown in colour. Acorn starch films isolated through enzymatic method were browner.

Keywords: acorn fruits, starch, films, isolation methods, contact angle.

Introduction
Acorn fruits from Quercus suber (QS) and Quercus rotundifolia (QR) are important forestry resources in the Central and Southern regions of Portugal, but they can also be important in Greece, Italy and France (Grove, Rackham, 2001). Most of the fruit production goes to animal feeding, mainly of Iberic pigs. These fruits are also consumed in other European countries (Rakic et al., 2006), and there are many different kinds of commercially available processed acorn products, including breads, cakes, soups, snacks, noodles and jelly, which are comprised principally of acorn flours (Kim, Yoo, 2009).

Starch as a natural component, contributes to the characteristic properties of food products such as texture, viscosity, gel formation, adhesion, binding, moisture retention, film formation and product homogeneity in different products like sauces, puddings, confectionary, and a variety of low-fat products. Starch is the main component of acorn flour, 31.4% (Correia, Beirão-da-Costa, 2012), suggesting that these fruits can be a good alternative to conventional starch resources, such as cereals and tubers. However, the properties of starches are not only dependent on the starch source but also highly dependent on the history of the starch itself (Wichmann et al., 2007) as for instance the extraction procedures. It is known that extraction procedures affect both the chemical composition and physical properties of starch, which justify the interest of studying the most suitable one for each individual raw material.

Many uses of starch involve heating it in water, which leads to granule hydration, swelling, and at a high enough temperature, solubilisation of starch molecules. The most important starch properties, with respect to its utilisation, are often related to the properties of gelatinized pastes. The gelatinized starch has the property of forming gels, and when it is dehydrated, it forms a hard, transparent, bright, and resistant film. Starch biofilms are produced from gelatinisation and after retrogradation of starch.

Wettability may be a convenient parameter providing information on surface properties of starch gels’ surface. The wettability of a solid surface can be determined by a relatively simple method, measuring the so-called contact angle (Adamson, 1990). The study of starch gels’ wettability and their surface free energy may be useful for food science and pharmacology fields. In food technology, a better understanding of the surface properties of gels can be useful in developing new food products and food components. In pharmacology, wettability can help to explain the drug delivery systems and mucosa adhesion (Bialopiotrowicz, 2003). Furthermore, an intense search for new renewable sources to produce edible and biodegradable materials is observed. Edible and biodegradable natural polymer films offer alternative packaging with lower environmental costs. The main renewable and natural biopolymer films are obtained from polysaccharides, lipids and proteins. Furthermore, starch is considered one of the most promising raw materials for developing biodegradable plastic to reduce the environmental impact of plastic wastes, especially from packaging (SinhaRay, Bousmina, 2005).

The objective of this work was to study acorn starch films colour characteristics, their wettability and their surface free energy, important parameters for food and non-food industries.
Materials and Methods

Sampling

Quercus suber Lam. (QS) and Quercus rotundifolia Lam. (QR) acorns were collected in “montados”, located in Idanha-a-Nova (Centre East of Portugal). The acorns were harvested at full maturity. Three sets of 2 kg were randomly collected for each species. Preparation of acorn fruits was performed as described by Correia and Beirão-da-Costa (2012).

Starch isolation methods

Starch was isolated similarly as from sorghum flours following two methods (Correia, Beirão-da-Costa 2012):

- Alkaline pH using successively three sieves (A)
  - The flours (120 g) were soaked in 2 volumes of 0.25% NaOH at 5°C for 24 hours. Suspensions were homogenised and screened through a 180 μm sieve. The procedure was then repeated twice. The precipitate was screened successively in 75 and 53 μm sieves. The mixture was centrifuged in a Universal centrifuge (Hettich Zentrifugen Company, Germany) at 800 g for 15 minutes, the mucilaginous layer was scraped away and the precipitate was then suspended in water. This last step was repeated twice. Isolated starches were dried for two days at 40°C in a FD 115 Binder ventilated drying chamber (with an air flow of 300 m³ per hour).
- Enzymatic method (E)
  - Protease from Aspergillus oryzae was purchased from Sigma Chemical Co. One unit of protease was defined as the amount of enzyme that liberated 1.0 μmol of tyrosine per minute from casein at pH 7.5 at 37°C. The protease was added (900 units) to 120 g individual flours. Water was added (360 mL) and the slurry was adjusted to pH 7.5 (with 0.1 M NaOH or 0.1 M HCl). The slurry was incubated at 37°C for 2 hours. The slurry was then centrifuged in the same conditions as previously mentioned. The starch fraction was suspended, washed with water (200 mL) and filtered through a 53 μm sieve. The filtrate was centrifuged. The supernatant and tailings were discarded and the starch dried as reported above. The yields and starch purities are shown in Table 1.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Isolation method</th>
<th>Yield, %</th>
<th>Purity, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>QR</td>
<td>Alkaline</td>
<td>88.5 a</td>
<td>98.1 a</td>
</tr>
<tr>
<td></td>
<td>Enzymatic</td>
<td>86.9 b</td>
<td>97.6 a</td>
</tr>
<tr>
<td>QS</td>
<td>Alkaline</td>
<td>87.6 a</td>
<td>98.0 a</td>
</tr>
<tr>
<td></td>
<td>Enzymatic</td>
<td>85.4 b</td>
<td>97.8 a</td>
</tr>
</tbody>
</table>

Table 1

Yield and purity of starches isolated from acorn flours through alkaline and enzymatic methods

Starch gels and preparation of their films

Starch gels and preparation of their films of 1, 2 and 3% were obtained from the isolated acorn starches, according to Silva et al. (2007). Two hundred milliliters of water suspension of a proper amount (e.g. 1, 2 and 3%) of dry starch powder were poured in a small (250 mL) stainless steel reactor vessel. The vessel was immersed in a thermostated bath at 85°C and its content was continuously stirred mechanically. After reaching 85°C, the heating continued during 40 minutes at continuous stirring. The obtained gel was cold, and 15 mL of gelatinised starch was placed in a plastic Petri dish, with 110 mm of diameter and 15 mm of thick. The Petri dish was placed in a dry oven with controlled temperature, at 25°C for 18 hours (the time necessary to remove the excess of water).

Contact angle measurements

Contact angles were measured on dry starch gel films. The contact angle for MilliQ water (Millipore, Molsheim, France), formamide pure Fluka AG (Switzerland), ethylene glycol p.a. POCh Gliwice (Poland), and glycerol p.a. POCh Gliwice (Poland) was determined on acorn gel films. Contact angle measurements were carried out by using a contact angle instrument, Contact Angle System OCA20 (Dataphysics, Germany) with multiple portions, and the Sessile and Captive drop method was applied. A 0.1 mL droplet of water (or the other used liquids) was placed on the sheet surface and the image of the drop was captured by a CCD digital video camera. The contact angles were measured on both sides of the drop and averaged. Samples of 30 mm of length and 5 mm of width were applied on a glass surface using double-sided adhesive tape. At least 30 measurements were done for each experiment. The measurements were made at room temperature in a closed box.

Colour parameters of acorn starch films

The colour of all gel films was measured using a handheld tristimulus colorimeter (Chroma Meter-CR-400). The parameters measured were the brightness L*, which varies between 0 and 100 (from black to white, respectively), and the coordinates of opposed colour: a* and b*, which vary from −60 to +60, where the a* assumes negative values for red and positive values for green and positive values for blue and positive for yellow. From L* a* b*, the hue angle (h°) and the chroma (c*) and hue angle (h°) were determined. Chroma or saturation, c* (0–60), measures how dull / vivid the object colour is; hue angle, h° (0–360°), expresses the characteristic/dominant colour (0 – red / purple; 90 – yellow; 180 – bluish / green). Films colour measurements were taken by putting films on white paper. The total colour change (ΔE), was the parameter considered for the overall colour difference evaluation, between a sample and the reference fruit (without storage, designated with an index 0):

\[ ΔE = \sqrt{(L_0 - L)^2 + (a_0 - a)^2 + (b_0 - b)^2} \]  

At least 30 measurements were done for each experiment.

Statistical analysis

The obtained results were subject to a one-way analysis of variance (ANOVA) test using the Statistic® vs 8
software. The separation of means or significant difference comparison of all parameters was tested by the Tukey’s HSD test. The level of significance used for all the statistical tests was 95%.

Results and Discussion

The isolation methods lead to acorn starches with high purity and yields (Table 1), and the alkaline method was considered to be the better one, mainly because of the higher yields. Considering the experimental conditions for film formation, the 3% gel concentration was the only sample with good results, producing films without breakage. The films showed different colour characteristics depending on the starch source and the isolation method (Figure 1 and Table 1).

![Figure 1. Appearance of acorn starch films(S)](image)

A – Starch alkaline isolation method, E – Starch enzymatic isolation method

The QS_A film seen to be the most distinguished one (Figure 1). The colorimetric results showed that the starch isolation method influences the films colour characteristics, but in different ways. The acorn films presented high transparency and predominant yellow colour (Table 2). QS films presented high absolute values of a* parameter, meaning that they assumed green colour. The QR films formed from starch isolated through enzymatic method were more vivid than the others. The encountered colour difference might be classified according to Drlange (1994) as great (ΔE between 6.0 and 12.0). The results showed that either for the variety QS_A, which corroborate the image showed in Figure 1.

![Table 2: Colour parameters of sorghum and corn films](image)

Means sharing the same letters in column are not significantly different from each other (Tukey’s HSD test, p<0.05).

![Figure 2. Contact angle (CA) and surface energy (SE) of acorn starch films](image)

E–Enzymatic method, A–Alkaline method

The contact angle of the biofilms formed from sorghum starch presented higher values than commercial corn starch (46°) (Gonçalves et al., 2012), meaning that the acorn films surface is more hydrophobic, with less water affinity (Fig. 2). It could be noticed that the starch isolation method did not affect significantly this characteristic. The surface energy was greatly influenced by the isolation method but differently and oppositely for QS and QR. Generally all the films presented low (or intermediate values of surface energy, when compared to other tested material, such as sorghum (38.0 and 40.7, respectively for alkaline and enzymatic isolation method) and commercial corn (90.0) starches (Gonçalves et al., 2012) which mean that acorn starches presented lower or intermediate intramolecular interactions forces. This characteristic is lower for QS_E and QR_A. The results also showed that the interaction forces dispersed and polar part are quite different between species and between isolation methods. For all samples the polar part was high, with the exception of QS films form from starch isolated through enzymatic method (Fig. 3) and this could be related with the lower values for surface free energy (combination of dispersed and
polar interactions forces). It was also noticed that for QS films produced from starch isolated from enzymatic method the dispersed component was higher.

Acorn films presented a hydrophobic surface with low water affinity. Moreover, the surface energy was low / intermediate which means that acorn starch presented weak intramolecular interaction forces. For all samples the interaction force polar part was high, with the exception of QS films from starch isolated through enzymatic method, which could be related with the lower values for surface free energy.

Acknowledgment
The authors thank the Portuguese Foundation for Science and Technology (FCT) and Research Centre CI&DETS for financial support (project PEst-OE/CED/UI4016/2014).

References
EFFECT OF DIFFERENT TYPES OF HEAT TREATMENT ON INVERTAZE ACTIVITY IN HONEY

Fredijs Dimins¹, Velga Mikelsones¹, Peteris Kuka¹, Arturs Niklavs Jefremovs²

¹ Department of Chemistry, Faculty of Food Technology, Latvia University of Agriculture, Liela Street 2, Jelgava, Latvia, e-mail:fredisd@tvnet.lv
² Jelgava Gymnasium Spidola, Matare Street 30, Jelgava, Latvia

Abstract
Honey is important foodstuff, which is often consumed fresh or used in other meals. Liquid honey is more demanded in market, so solid honey often is heated in order to melt it. One of the most traditional ways to heat food and its substances is by heating it in the microwave oven and in the thermostat, while changing microwave oven power, heating duration and heating temperature in thermostat. The aim of the research is to study and to compare, how invertase activity in honey is affected by heating it in the microwave oven and in the thermostat. Enzymes – sensitive enzymes is invertase.

The method of research is to study and to compare invertase activity in honey, heated in the microwave oven and in the thermostat. Enzymes – sensitive enzymes is invertase. The method of research is to study and to compare, how invertase activity in honey is affected by heating it in the microwave oven and in the thermostat.

Analyzing different honey samples, the following results were obtained. When honey samples were heated in 450 W microwave oven, invertase activity significantly decreased after 20 seconds. After 25 seconds invertase activity decreased 5.8 times, after 30 seconds – 12.5 times. After heating honey samples in thermostat in 60 °C for 120 minutes, invertase activity decreased only twice. Summarizing research results, we concluded, that invertase activity changes are significantly higher in honey samples, heated in the microwave oven, than honey samples, heated in the thermostat.

Keywords: Honey, invertase activity, thermal treatment, microwave.

Introduction
Food often gets the thermally treated to heat it. Food is heated faster in microwaves than in usual oven. Honey is one of most used foodstuff. Honey in food is used both raw and in confectionery products. In order to better sell honey it’s often heated. A small amount of honey can be quickly heated using microwaves. Microwaves are electromagnetic radiation in the wavelength range between 30 cm to 1 mm (1 GHz–300 GHz), by other data 1 m – 1 mm (300 MHz–300 GHz). Microwaves are essentially short radio waves and in the past they were not separated from radio waves; they are produced by high-frequency power generator, called magnetron (Electromagnetic Energy and Microwaves, 2013).

Microwave radiation intensity depends on the tubes that are used in device. There are separated high power and low power microwave radiation sources. Microwave transmitters and receivers are similar to radio wave transmitters and receivers. Microwave transmitter tubes contain tuned circuit, which is able to perceive or transmit certain frequency bands. Product heating in microwave oven happens when high-frequency electromagnetic wave energy is transformed in thermal energy; in result products cooking time is two to three times lower than usual. Microwave principle of operation is to broadcast non-ionizing microwave radiation, usually 2.45 GHz, through food. Substances such as water, grease and other receive energy from microwaves in process called dielectric heating. Many molecules, including water are polar – with a single positive charge on one side and negative charge on the other. Transmitting microwave radiation, water molecules continuously rotates, relative to the prevailing magnetic field that varies 2.45 million times per second. As rotation speed of water molecules increases, product temperature increases as well (Sapunov, 2007; Electromagnetic Energy and Microwaves, 2013). Affecting food, such as honey, with microwaves denaturates proteins within it, changes vitamin content and also reduces the activity of enzymes (Sapunov, 2007; Hendrickson, 2011). Honey is losing its biological and antibacterial activity and many of its component volatiles.

Some of the honey quality and biological activity indicators are the enzymes. Enzymes are complex proteins that catalyze reactions within cells. Enzymes have huge impact in human life processes. Honey naturally preserves small amounts of enzymes. Honey mostly contains enzyme amylase, glucose oxidase, catalase and invertase, and their contents can vary depending on the region in which honey is collected (Delaplane, Mayer, 2000).

Enzyme functions and catalyzed reactions determine honey composition and pH level and antibacterial properties.

One of honey quality indicator and thermal treatment indicator is enzyme invertase (Karabournioti, Zervalaki, 2001).
Table 1

Enzyme catalyzed reactions in honey
(Honey enzymes, 2014)

<table>
<thead>
<tr>
<th>Enzyme in honey</th>
<th>Substratum</th>
<th>Reaction products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invertase</td>
<td>Sucrose</td>
<td>Glucose and fructose</td>
</tr>
<tr>
<td>Glucose oxidase</td>
<td>Glucose</td>
<td>Gluconic acid and hydrogen peroxide</td>
</tr>
<tr>
<td>Catalase</td>
<td>Hydrogen peroxide</td>
<td>Water and oxygen</td>
</tr>
<tr>
<td>Amylase (Diastase)</td>
<td>Starch</td>
<td>Dextrose, maltose and glucose</td>
</tr>
</tbody>
</table>

Invertase is an enzyme which splits sucrose into glucose and fructose. Invertase activity is expressed as the invertase number or invertase units.

Figure 1. Reaction catalyzed by invertase
(Garret, Grisham, 2005)

Invertase unit is 1 micromole of substrate that is cleaved within 1 minute. Invertase number is the amount in grams of sucrose, which within 1 hour is hydrolyzed by invertase in 100 g honey (1 IN=7.344732 IU kg⁻¹). According to the European Commission's recommendations, invertase number should be higher than 10 (individual honey samples greater than 4) (Bogdanov, 2009; Honey Quality and International Regulatory Standards, 2014).

The aim was to find out how honey treatment in microwave heating in a thermostat affect invertase activity and hence the quality of honey and its biological properties. To achieve the goal invertase activity were determined in honey samples. Honey samples were heated in a thermostat, and also treated in microwave oven.

Materials and Methods

In research buckwheat flower honey samples collected in 2013 in Jelgava District Sesavas parish were analyzed. The analysis was performed at the Department of Chemistry of LUU laboratories. Invertase activity in honey samples were determined spectrophotometrically using Jenway 6300 spectrophotometer.

Research of microwaves influence was analysed, using Samsung microwave oven. p-Nitrophenyl-α-D-glucopyranoside (pNPG) is used as a substrate for the determination of the invertase number in honey. pNPG is split into glucose and p-nitrophenol by α-glucosidase (invertase, sucrase).

By adjusting the pH-value to 9.5 the enzymatic reaction is stopped and at the same time nitrophenol is transformed into the nitrophenolate anion, which corresponds to the amount of converted substrate and is determined spectrophotometrically at 400 nm (Bogdanov, 2009).

Figure 2. Overall determination scheme of invertase activity
Research on microwave radiation impact were made by treating honey samples in microwave oven 10 s using 180 W, 300 W, 450 W, 600 W in 800 W high power microwave. According from the results, the optimal choice of microwave intensity was chosen. The thermostat heating temperature was selected by heating the honey samples for 1 h in 50 °C, 60 °C and 70 °C. Then, according from the results the optimal temperature was used in subsequent experiments. The general methodology for determining invertase activity is shown in Figure 2.

Results and Discussion
Microwave oven power influence on invertase activity in honey is shown in Figure 3.

Figure 3. Changes of invertase activity in various microwave oven powers

Honey samples, using 180 W, 300 W, 450 W, 600 W, 800 W microwave oven power, were treated 10 s. As can be seen from the results of Figure 3, increasing microwave power, reduce the invertase activity in honey samples. Raw honey sample have invertase activity of 85±8 IU, which indicates honey sufficiently high enzyme activity and thus biological - functional properties. This is consistent with the recommendations of the International Honey Commission (IN≥10; IU≥73.45). To study microwave radiation duration effect on invertase activity, had to opt for microwave power. It may be more accurate and more appropriate to do this by studying the dynamics of invertase activity decay times by reference to the fresh honey invertase activity.

Figure 4. Correlation between decrease of invertase activity and microwave oven powers

As can be seen from the Figure 4 data, a significant decrease in invertase activity occurs from 450 W microwave oven power. Therefore, studies on the effects of microwave radiation on the invertase activity, depending on the microwave radiation exposure time, is carried out using 450 W microwave power.

Figure 5. Changes of invertase activity depending on the duration of exposure to microwave radiation

Figure 5 shows that by treating honey samples in a microwave oven power of 450 W, significant changes of invertase activity started to happen after 20 seconds. After 25 seconds of treatment in a given microwave oven power invertase activity decreases 5.8 times, but when treated for 30 seconds – 12.5 times. It means that significant honey quality changes occur, thus, protein denaturation occurs; it worsens the healing and anti-bacterial properties.

In order to judge the effect of microwave radiation, honey samples were also heated in thermostat using different heating temperatures and heating lengths. In order to assess the heating effect of temperature on invertase activity in honey were heated for 1 hour at 50 °C, 60 °C and 70 °C.

Figure 6. Effect of the heating temperature on invertase activity in honey

As can be seen from Figure 6, increasing the heating temperature, the invertase activity decreases. In order to characterize the duration of the heating effect of honey on honey invertase activity in honey and correctly select the heating temperature, the
invertase activity change dynamics were characterized by invertase activity decay times relative to the fresh honey (uncooked) invertase activity.

**Figure 7. Correlation between decrease of invertase activity and heating temperature**

As can be seen from Figure 7, invertase activity starts to decrease significantly from 50 °C temperature. In order to characterize the invertase activity of correlation between the duration of heating, the temperature of 60 °C was chosen because at this temperature for invertase activity has been greatly reduced. Invertase activity of correlation between the duration of the heat treatment is shown in Figure 8.

**Figure 8. Correlation between invertase activity and the heating time of honey**

As you can see from the Figure 8, honey invertase activity changes are not so great when compared to the invertase activity changes in honey samples heated in the microwave.

This is best demonstrated when comparing change in invertase activity decay times in a microwave oven and in a thermostat treated honey samples.

**Figure 9. Decrease of invertase activity depending on heating time in microwave oven**

As can be seen from the Figure 9 invertase activity changes in the microwave oven heated honey samples are insignificant if honey is heated less than 20 s, on the other hand, from the 20 s invertase activity changes occur very rapidly. On the other hand, if we compare the thermostat heated honey samples, they invertase activity gradually decreased from the beginning of the heating. However the invertase activity decrease is lower than in microwave oven heated honey. For example, at the end of experiment, in thermostat 2 hours heated honey invertase activity decreased 2 times (Fig. 10), however in 450 W microwave oven it decreased 5.86 times after 25 s and 12.52 times after 30 s heating.

That means if honey needs to be heated then it should be done using conventional heating. In microwave oven honey can be heated very short time period, and if honey is heated for 30 s or longer, then honey can completely lose its quality.

**Figure 10. Decrease of invertase activity depending on heat treatment**

**Conclusions**

Treating of the honey samples in a microwave oven, invertase activity changes occur much faster than heating it in an oven. Treating of the honey samples in Samsung microwave oven significant invertase activity changes occurs if microwave oven power is higher than 450 W.

Treating of the honey samples in a microwave power of 450 W, a significant invertase activity changes occur after 20 s. After 25 s of treatment in a given power invertase activity decreases 5.8 times, while treating the 30 s – 12.5 times.

Heating of the honey samples in a thermostat at 60 °C for 2 h, invertase activity gradually decreases twice. Treating of the honey samples in microwave oven significantly reduces the activity of biologically active substances.
In thermostat heated honey samples, significant invertase activity changes occurs if heating temperature exceed 50 °C. Enzymes and other proteins denaturates – lose activity, if they are heated in thermostat or treated in microwave radiation. The invertase number of analyzed honey samples (11.57) indicates the high quality of honey.

References
COMPARISON OF THREE DIFFERENT TECHNIQUES FOR EXTRATION OF VOLATILES FROM PISTACHIO NUTS

Perihan Kendirci, Tomris Altug Onogur
Food Engineering Department, Engineering Faculty, Ege University, Bornova, Izmir, Turkey, e-mail: perihan.kendirci@ege.edu.tr

Abstract

Pistachio nut (Pistacia vera L.) is one of the popular tree nuts of the world and is widely cultivated in saline, dry and hot areas of the Middle East, Mediterranean countries and United States. Iran, USA, Syria and Turkey are the main producer countries of this product. Pistachio nut is widely consumed as a raw or roasted ingredient of many desserts, ice creams, cakes, pastry and in the production of some sausages. The aim of the present study was to determine the appropriate technique for extraction of volatiles from pistachio nuts. Uzun variety which is cultivated in the South-East Region of Turkey was used as the material of the study. Volatiles were extracted with three different extraction techniques – Liquid-Liquid Solvent Extraction (LLSE) at 85±2 °C for 6 h by using ethanol and hexane as solvents, Headspace extraction (HS) at 100°C for 1 h and Solid Phase Micro Extraction (SPME) at 100 °C for 1 h by using DVB/Car/PDMS fiber. Obtained extracts were analyzed by Gas Chromatography/Mass Selective detector (GC/MS). The total number of volatiles extracted by LLSE, HS and SPME techniques were 34, 41, 25, respectively; and the percentage of volatiles identified by these techniques were found to be 67.36%, 72.04%, 92.91%, respectively. Since more than 90% of volatiles could be identified by SPME which also showed high repeatability, it could be recommended as the most appropriate technique for extraction of volatiles from pistachio nuts.

Keywords: pistachio nut, extraction of volatile components, GC/MS analysis.

Introduction

Pistachio nut (Pistacia vera L.) is one of the popular tree nuts of the world and is widely cultivated in saline, dry and hot areas of the Middle East, Mediterranean countries and United States (Maskan, Karatas, 1999; Kashani-Nejad et al., 2003). According to the FAOSTAT Iran, USA and Turkey are the main producer countries of pistachio nuts (Anon, 2014a). One of the major cultivar of pistachio nuts grown in Turkey is Uzun and it is mainly preferred for the production of baklava and nut paste because of its special green kernel colour, flavour and texture (Balta, 2002; Gamli, Hayoglu, 2007). Different kind of extraction techniques have been studied to determine the volatile compound of different types of nuts such as solvent extraction technique for chestnuts (Morini, Maga, 1995), almonds (Cantalejo, 1997; Vazquez-Araujo et al., 2008), peanuts (El-Kayati et al., 1998; Ku et al., 1998), hazelnuts (Kiefl, Schieberle, 2013) headspace (HS) technique for peanuts (Young, Hovis, 1990; Braddock et al. 1995; Burroni et al., 1997; Ku et al. 1998, Alasalvar et al., 2003); and solid phase micro extraction (SPME) technique for peanuts (Abegaz et al., 2004; Krist et al., 2004. Although the flavour of pistachio nuts has wide appeal, there is not enough study about this topic. Soliman et al (1981) studied the volatiles of roasted pistachio nuts by using vacuum carbon dioxide distillation of acetone extracts and identified the volatiles as pyrazines, pyrrols, aldehydes and some others by using GC/MS. In another study conducted by Kendirci and Altug (2011) the volatile compounds of different varieties of fresh pistachio nuts were extracted by using SPME-GC/MS and it was found that volatiles of fresh pistachio nuts were mainly composed of terpenes like α-pinene, α-terpinolene, limonene, β-myrcene. In another study where volatiles of roasted pistachio nut were analysed, pyrazines, aldehydes, acids and some other compounds were detected (Acena et al. 2011).

In this study, the volatile components of Uzun variety of pistachio nuts grown in Turkey were extracted using three different extraction methods to determine the appropriate technique. For this purpose, Liquid-Liquid Solvent Extraction (LLSE), Headspace extraction (HS) and Solid Phase Micro Extraction (SPME) was applied to the pistachio nuts. Obtained extracts were analyzed by Gas Chromatography equipped with Mass Selective Detector (GC/MS).

Materials and Methods

Materials

Uzun variety of pistachio nuts (Pistacia vera L.) were obtained from Pistachio Nut Research Institute in Gaziantep, Turkey at the beginning of the harvest season in September 2005.

Liquid Liquid Solvent Extraction (LLSE)

The method described by Heath and Reineccius (1986) was applied to the samples as a LLSE technique. For this purpose, 40 g of ground pistachio nuts were transferred to the sample tube of LLSE apparatus with the help of 100 mL of ethanol; while 125 mL of hexane was in the collector conical flask (Figure 1).

Figure 1. LLSE apparatus
Conical flask was placed in to the water bath at 85±2 °C and extraction was continued for 6 h. Hexane extract was concentrated to 10 mL at room temperature by the help of nitrogen gas. 5 μL of extract was injected in the GC/MS (Hewlett Packard, USA).

**Headspace Extraction (HS)**

HS extraction was performed by modifying the methods suggested by Young, Hovis (1990), Burroni et al. (1997) and Ku et al. (1998). For this purpose 8 g of ground sample was weighed into a screw top amber vial with black viton septa which was placed into a block heater at 100 °C. After 60 min, 1 mL of headspace was injected to the GC/MS device.

**Solid Phase Micro extraction (SPME)**

SPME procedure was performed by using a manual SPME device and extractions were carried out by using a 50 / 30 μm DVB/Carboxen/PDMS stable-Flex fiber as suggested in Abegaz et al. (2004) and Krist et al. (2004). 8 grams of dehulled and ground sample was weighed into a screw top amber vial with black viton septa which was placed into a block. SPME fibre was immersed to the headspace of the vial and the volatiles were collected for 60 min at 100 °C. After sampling, the SPME fiber was removed from the vial and introduced onto the injection port of GC/MS.

**GC/MS Analysis**

The volatiles were separated using HP-5MS 5% phenylmethylcyloxane column (30.0 m×0.25 mm×0.25 μm film thickness) that was attached to a Hewlett Packard Model HP-6890 gas chromatograph equipped with a HP 5973 Mass Selective detector. The operating conditions of the GC were as follows: injector temperature of 250 °C, splitless mode, carrier gas of helium (with an inlet flow rate of 1 mL min⁻¹). The temperature gradient used began at 60 °C for 1 min, then was raised to 260 °C at 5 °C min⁻¹ and held at this temperature for 45 min. Mass spectra were generated at 70 eV. The mass selective detector was scanned from 30 to 350 at 1 scan s⁻¹. Identification of the volatile compounds was achieved by comparing retention times with those in the MS library (NIST and WILEY).

**Interpretation of the Results**

The analyses were applied as triplicate for each technique, and the standard deviations from the mean values were calculated and shown on tables.

**Results and Discussion**

The chromatographs of the volatiles extracted from the pistachio nuts by using LLSE, HS and SPME techniques are shown on Figures 1–3; list of the volatile compounds are given in Tables 1–3.

As it can be seen from Table 1 and Table 4, 34 volatile compounds were extracted from pistachio nuts using LLSE technique. 32.62% of the total peak area of the volatiles could not be identified, while 35.41% them were determined as 9-octadecanoic acid isomers having a fatty character (Anon 2014b). The percentage of α-pinene which is one of the major volatiles of the pistachio nuts (Kendirci, Altug, 2011) was detected to cover only 0.16% of the total peak area. The repeatability of the technique was determined to be low since the standard deviations of the results were high from Table 1.
The volatile compounds extracted by using LLSE technique

<table>
<thead>
<tr>
<th>Peak</th>
<th>t_r (min)</th>
<th>Volatile compound</th>
<th>Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.60</td>
<td>α-pinene</td>
<td>0.16±0.28</td>
</tr>
<tr>
<td>2</td>
<td>12.4</td>
<td>1-dodecene</td>
<td>0.57±0.49</td>
</tr>
<tr>
<td>3</td>
<td>12.58</td>
<td>dioctane</td>
<td>0.47±0.41</td>
</tr>
<tr>
<td>4</td>
<td>17.87</td>
<td>1-tetradecane</td>
<td>1.30±0.97</td>
</tr>
<tr>
<td>5</td>
<td>18.02</td>
<td>tetradeacne</td>
<td>0.37±0.53</td>
</tr>
<tr>
<td>6</td>
<td>21.34</td>
<td>2,6-bis(1,1- dimethyl)ethy1)-4-phenol</td>
<td>1.81±0.97</td>
</tr>
<tr>
<td>7</td>
<td>22.89</td>
<td>1-hexadecane</td>
<td>1.13±1.07</td>
</tr>
<tr>
<td>8</td>
<td>27.44</td>
<td>5-octadecene (E)</td>
<td>0.59±0.57</td>
</tr>
<tr>
<td>9</td>
<td>29.07</td>
<td>hexamethyl pyranownane</td>
<td>0.04±0.07</td>
</tr>
<tr>
<td>10</td>
<td>31.19</td>
<td>Unidentified</td>
<td>0.04±0.08</td>
</tr>
<tr>
<td>11</td>
<td>31.63</td>
<td>Hexadecanoic acid</td>
<td>1.10±0.98</td>
</tr>
<tr>
<td>12</td>
<td>32.28</td>
<td>n-hexadecanoic acid</td>
<td>0.62±1.07</td>
</tr>
<tr>
<td>13</td>
<td>32.85</td>
<td>9-octadecanoic acid (Z), isomer</td>
<td>1.15±1.07</td>
</tr>
<tr>
<td>14</td>
<td>33.40</td>
<td>5-eicosene</td>
<td>0.35±0.61</td>
</tr>
<tr>
<td>15</td>
<td>33.74</td>
<td>9-octadecanoic acid (Z), isomer</td>
<td>0.63±0.34</td>
</tr>
<tr>
<td>16</td>
<td>34.96</td>
<td>ethyl oleate</td>
<td>10.17±7.50</td>
</tr>
<tr>
<td>17</td>
<td>35.70</td>
<td>9-octadecanoic acid (Z), isomer</td>
<td>19.46±26.69</td>
</tr>
<tr>
<td>18</td>
<td>36.51</td>
<td>9-octadecanoic acid (Z), isomer</td>
<td>1.13±0.78</td>
</tr>
<tr>
<td>19</td>
<td>37.40</td>
<td>Unidentified</td>
<td>1.26±0.60</td>
</tr>
<tr>
<td>20</td>
<td>39.01</td>
<td>9-octadecanoic acid (Z), isomer</td>
<td>0.07±0.12</td>
</tr>
<tr>
<td>21</td>
<td>39.72</td>
<td>9-octadecenol</td>
<td>0.60±0.98</td>
</tr>
<tr>
<td>22</td>
<td>40.00</td>
<td>Unidentified</td>
<td>0.14±0.24</td>
</tr>
<tr>
<td>23</td>
<td>40.41</td>
<td>9,17-octadecadienol (Z)</td>
<td>13.74±8.86</td>
</tr>
<tr>
<td>24</td>
<td>40.8</td>
<td>Unidentified</td>
<td>0.52±0.91</td>
</tr>
<tr>
<td>25</td>
<td>41.17</td>
<td>Unidentified</td>
<td>2.91±3.12</td>
</tr>
<tr>
<td>26</td>
<td>41.40</td>
<td>1,12-tridecadiene</td>
<td>1.43±2.47</td>
</tr>
<tr>
<td>27</td>
<td>43.45</td>
<td>Unidentified</td>
<td>1.38±0.70</td>
</tr>
<tr>
<td>28</td>
<td>45.42</td>
<td>9-octadecanoic acid (Z), 3-hydroxypropyl ester</td>
<td>2.47±4.28</td>
</tr>
<tr>
<td>29</td>
<td>47.31</td>
<td>9-octadecanoic acid (Z), isomer</td>
<td>6.55±7.36</td>
</tr>
<tr>
<td>30</td>
<td>53.73</td>
<td>9-octadecanoic acid (Z), isomer</td>
<td>3.95±2.08</td>
</tr>
<tr>
<td>31</td>
<td>58.77</td>
<td>Unidentified</td>
<td>0.27±4.28</td>
</tr>
<tr>
<td>32</td>
<td>61.05</td>
<td>Unidentified</td>
<td>4.15±7.18</td>
</tr>
<tr>
<td>33</td>
<td>76.08</td>
<td>Unidentified</td>
<td>2.97±2.38</td>
</tr>
<tr>
<td>34</td>
<td>84.28</td>
<td>Unidentified</td>
<td>15.03±26.03</td>
</tr>
</tbody>
</table>

41 volatiles could be extracted from the pistachio nuts and the repeatability of HS technique was higher in comparison with LLSE technique (standard deviations are lower than LLSE) (Table 2). On the other hand, 27.90% of the total extracted volatile compounds could not be identified and the percentage of α-pinene was found to be only 0.16% (Table 4). Table 3 shows that 25 volatile compounds were extracted from samples by using SPME technique.

---

**Table 2**

The volatile compounds extracted by using HS technique

<table>
<thead>
<tr>
<th>Peak</th>
<th>t_r (min)</th>
<th>Volatile compound</th>
<th>Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.36</td>
<td>hexanal</td>
<td>8.20±0.67</td>
</tr>
<tr>
<td>2</td>
<td>4.20</td>
<td>2-ethyl-3-vinlyloxirane</td>
<td>0.18±0.25</td>
</tr>
<tr>
<td>3</td>
<td>5.04</td>
<td>heptanoen</td>
<td>2.32±0.28</td>
</tr>
<tr>
<td>4</td>
<td>5.66</td>
<td>α-pinene</td>
<td>0.73±1.04</td>
</tr>
<tr>
<td>5</td>
<td>6.05</td>
<td>Unidentified</td>
<td>0.24±0.34</td>
</tr>
<tr>
<td>6</td>
<td>6.35</td>
<td>2-heptanen, (E)</td>
<td>4.56±0.31</td>
</tr>
<tr>
<td>7</td>
<td>6.85</td>
<td>heptanol</td>
<td>0.32±0.45</td>
</tr>
<tr>
<td>8</td>
<td>7.14</td>
<td>1-octen-3-ol</td>
<td>9.29±0.41</td>
</tr>
<tr>
<td>9</td>
<td>7.39</td>
<td>octanal</td>
<td>2.54±0.38</td>
</tr>
<tr>
<td>10</td>
<td>8.96</td>
<td>octenal</td>
<td>0.89±0.04</td>
</tr>
<tr>
<td>11</td>
<td>9.58</td>
<td>pentylcyclopropane</td>
<td>0.92±0.12</td>
</tr>
<tr>
<td>12</td>
<td>10.15</td>
<td>nonanal</td>
<td>9.60±0.95</td>
</tr>
<tr>
<td>13</td>
<td>11.82</td>
<td>2-nonenal</td>
<td>1.05±0.07</td>
</tr>
<tr>
<td>14</td>
<td>12.93</td>
<td>decanal</td>
<td>0.17±0.24</td>
</tr>
<tr>
<td>15</td>
<td>13.68</td>
<td>Unidentified</td>
<td>0.31±0.43</td>
</tr>
<tr>
<td>16</td>
<td>14.29</td>
<td>Unidentified</td>
<td>0.98±0.04</td>
</tr>
<tr>
<td>17</td>
<td>14.71</td>
<td>2-decanol (E)</td>
<td>7.91±0.07</td>
</tr>
<tr>
<td>18</td>
<td>15.70</td>
<td>2,4-decadial (E,E)</td>
<td>1.58±0.16</td>
</tr>
<tr>
<td>19</td>
<td>16.39</td>
<td>2,4-decadial</td>
<td>3.12±0.12</td>
</tr>
<tr>
<td>20</td>
<td>16.82</td>
<td>Unidentified</td>
<td>0.12±0.17</td>
</tr>
<tr>
<td>21</td>
<td>17.00</td>
<td>Unidentified</td>
<td>0.51±0.72</td>
</tr>
<tr>
<td>22</td>
<td>17.50</td>
<td>2-octenal</td>
<td>7.96±0.30</td>
</tr>
<tr>
<td>23</td>
<td>18.13</td>
<td>Unidentified</td>
<td>1.00±1.42</td>
</tr>
<tr>
<td>24</td>
<td>22.55</td>
<td>8-hexadecane (Z)</td>
<td>1.16±0.08</td>
</tr>
<tr>
<td>25</td>
<td>24.47</td>
<td>Unidentified</td>
<td>0.23±0.33</td>
</tr>
<tr>
<td>26</td>
<td>24.85</td>
<td>8-heptadecene</td>
<td>2.88±0.43</td>
</tr>
<tr>
<td>27</td>
<td>25.51</td>
<td>1-cloro tetradecane</td>
<td>0.27±0.39</td>
</tr>
<tr>
<td>28</td>
<td>27.62</td>
<td>7-hexadecimal (Z)</td>
<td>0.30±0.42</td>
</tr>
<tr>
<td>29</td>
<td>29.35</td>
<td>Unidentified</td>
<td>0.26±0.37</td>
</tr>
<tr>
<td>30</td>
<td>29.82</td>
<td>9-oxabicyclo[6.1.0]nonane</td>
<td>4.83±0.21</td>
</tr>
<tr>
<td>31</td>
<td>33.33</td>
<td>Unidentified</td>
<td>0.55±0.15</td>
</tr>
<tr>
<td>32</td>
<td>35.58</td>
<td>9-octadecanoic acid (Z)</td>
<td>3.78±5.34</td>
</tr>
<tr>
<td>33</td>
<td>36.54</td>
<td>Unidentified</td>
<td>6.87±0.05</td>
</tr>
<tr>
<td>34</td>
<td>37.45</td>
<td>E.E-10,12-hexadecadiene-1-ol acetate</td>
<td>0.12±0.17</td>
</tr>
<tr>
<td>35</td>
<td>39.83</td>
<td>9-octadecanoic acid (Z), isomer</td>
<td>0.41±0.59</td>
</tr>
<tr>
<td>36</td>
<td>40.51</td>
<td>9-octadecanoic acid (Z), isomer</td>
<td>0.97±1.38</td>
</tr>
<tr>
<td>37</td>
<td>40.76</td>
<td>9-octadecanoic acid (Z), isomer</td>
<td>2.49±1.31</td>
</tr>
<tr>
<td>38</td>
<td>41.46</td>
<td>9-octadecanoic acid (Z), isomer</td>
<td>2.56±1.91</td>
</tr>
<tr>
<td>39</td>
<td>46.95</td>
<td>Unidentified</td>
<td>1.28±1.81</td>
</tr>
<tr>
<td>40</td>
<td>47.33</td>
<td>Unidentified</td>
<td>2.02±2.85</td>
</tr>
<tr>
<td>41</td>
<td>53.94</td>
<td>Unidentified</td>
<td>13.53±4.38</td>
</tr>
</tbody>
</table>
Although the total number of the volatiles extracted by SPME were lower in comparison with the other two techniques, the percentage of unidentified volatiles were much lower (7.09%).
Additionally, it was determined that 69.78% of the total peak area of volatiles was composed of 9-octadecanoic acid isomers (Table 4) which gives fatty character (Anon, 2014b); while 7.79% was limonene and α-terpinolene which gives citrus character (Anon, 2014b), 5.64% was α-pinene which gives pine character (Anon, 2014b), 1.80% was β-myrcene which gives must, balsamic and spice characters (Anon 2014b), and 1.34% was 2-decenal and 2-octenal which give green, nut and fatty characters (Anon, 2014b) of pistachio nuts. Table 4 shows the proportion of volatile compound groups extracted from pistachio nuts by using three different extraction techniques. As it can be seen from the Table 4, among the extraction techniques examined in this study, SPME was the best technique for extracting the terpene groups of the volatiles which are expected to be one of the main volatile groups of pistachio nuts (Kendirci, Altuğ, 2011) while aldehydes were the main group of HS technique. Because of the high fat content of the pistachios, mainly fatty acids (47.30 %) were determined by LLSE technique (Table 4).

Conclusions
Among the three extraction technique studied in this study, SPME seems to be the best technique for the extraction of the volatile compounds of pistachio nuts. By this technique the major volatiles which make up pistachio nut flavour could be extracted, and the percentage of unidentified compound were detected to be low in comparison with the other techniques examined in this study

Acknowledgment
This study was financially supported by EBILTEM (E.U. Scientific Research and Technology Center) and AROMSA Ltd.

References
RHEOLOGY, TECHNOLOGICAL AND SENSORY CHARACTERISTICS OF FORTIFIED DRINK PRODUCTS WITH FIBERS

Ausra Simoneliene, Ernests Treciokiene, Giedre Lukosiusaite, Gediminas Vysniauskas, Ernests Kaspaviciute

Food Technology Department, Faculty of Technology and Landscaping, Kauno kolegija / University of Applied Sciences, Pramonės pr. 22, Kaunas, Lithuania, e-mail: ausra.simoneliene@go.kauko.lt

Abstract

Fruits, vegetables and grain all produce a complex carbohydrate known as fiber that plays an important role in overall health. As fiber passes through gastrointestinal system, it isn't absorbed or digested like protein or fat. Instead, it stays in intestines to produce soft, formed stools that pass easily through body. Fiber also combines with fluid to create a gel-like substance that helps lower the level of cholesterol and glucose in blood. To make yoghurt even more beneficial to health and to replace conventional stabilizers with newer, healthier ones a research has been carried out, during which conventional processing aids used to improve the consistency of yoghurt, were replaced with orange pulp, pectin, bamboo and cane fibers. This study evaluated the effect of the supplementation of the same dietary fiber on the syneresis, stability, pH, acidity, dry matter content, viscosity and sensory evaluation of yogurt, juices and juice drinks. Dry matter content evaluation results showed that all the fibers were able to increase the dry matter content of yoghurt, juices and juice drinks. Viscosity measurements confirmed that fiber improved the consistence of yogurt. Results indicate that the Citri-Fi fiber is an almost applicable ingredient for the design of new high value-added yoghurt, juices and juice drinks.

Keywords: dietary fiber, yoghurt, juice drinks, texture.

Introduction

Fruits, vegetables and grain all produce a complex carbohydrate known as fiber that plays an important role in overall health. As fiber passes through gastrointestinal system, it isn't absorbed or digested like protein or fat. Instead, it stays in intestines to produce soft, formed stools that pass easily through body. Fiber also combines with fluid to create a gel-like substance that helps lower the level of cholesterol and glucose in blood and dietary fiber intake on a daily basis to prevent obesity, atherosclerosis, heart diseases, gut cancer and diabetes (Granato et al., 2010, Ramage et al., 2014). High quantities of new minimally processed foods have appeared on the market in response to a growing demand for natural products that are perceived by consumers as healthier. Among them are beverages based on a mixture of fruit juices and milk fortified with vitamins, minerals and fiber (Renuka, 2009). These beverages are the most widely consumed functional foods. Today's consumers who being more health conscious are seeking products with greater health benefits and there is a great demand for “health foods”.

A major concern of the yoghurt industry is the production and maintenance of a product with optimum consistency and stability. Tamime and Robinson (2007) mentioned that factors known to improve consistency are increasing total solids, manipulation of processing variables and characteristics of starter culture. The addition of stabilizers (such as polysaccharides) improves body and texture, appearance and mouth feel and retards syneresis of yoghurts (Hussein et al., 2011). The addition of polysaccharides as a stabilizer in the manufacture of yoghurt is a common practice. Stabilizers are sometimes referred to as hydrocolloids and have two basic functions in yoghurt: the binding of water and improvements in texture.

For the development of new fermented milks, the influence of modifications in the milk base on texture, rheology and sensorial properties of products has been studied, concerning mainly the lipid content of milk (Santo et al., 2012a, 2012b), the total dietary fiber (DF) contents (Santo et al., 2013), and the addition of proteins to increase total solids (Marafon et al., 2011). Formulation of new food products with ingredients from fruit by-products rich in total DF has increased in recent years. Dietary fiber can be fractioned into two major groups of components, the water-insoluble and the water-soluble fraction. While the insoluble fraction stimulates the intestinal peristalsis, the soluble one promotes the selective growth of the indigenous microbiota, acting as a prebiotic. Therefore it is healthier to consume the total dietetic fiber, instead of just its prebiotic fraction. One of the promising fruit by-products is orange pulp fiber, which, in addition to its functional properties such as the reduction of cholesterol and glucose in blood serum. Moreover, orange pulp fiber enhanced the texture parameters of ice-cream (Crizel et al., 2013). Based on this background, the present study aimed to evaluate some important aspects of the rheology, spontaneous whey separation, acidity of fruit juice beverages (apple juice) and yoghurts enriched with different kinds of fiber.

Materials and Methods

Preparation of apple juice

Fibers were mixed with the apple juice after pasteurization 85 °C, in 200 mL pasteurized apple juice added 3 g fibers. By analogy, all tests have been carried out with different types of fiber: Potatoes fiber (Campus fiber 110), Orange pulp fiber (Citri-Fi 100). When fibers were placed in the juice, the mixtures were homogenized at 10000 rpm and left in the fridge for 24 hour at 4 °C.
Preparation of yoghurt

Four types of yoghurts were prepared. The fibers used for yoghurt preparation were used: sugar cane fiber (JustFiber SC 200), bamboo fiber (JustFiber BFC 40), Orange pulp fiber (Citri-Fi 100). For the analysis, each yoghurt type was prepared in duplicate in two independent batch fermentations (N=4). 0.5% and 1% (in the final yoghurt) concentrations of fibers (Table 1) were dissolved individually in the 500 mL amount of milk, homogenized, and heated to 85 °C for 5 min. The flasks containing 500 mL of the heat treated skim milk base were inoculated with yoghurt starter cultures (YO-MIX LYO DUC, Danisco, Germany). Afterwards, the flasks with the samples were transferred to a water bath at 42 °C. At pH 4.5, fermentation was stopped and the flasks were cooled to 6 °C in an ice bath and stored in refrigerator at 4 °C.

Table 1

<table>
<thead>
<tr>
<th>Fibre</th>
<th>Appearance</th>
<th>Total dietary fiber, %</th>
<th>Ash, %</th>
<th>pH (10% suspension)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar cane fiber</td>
<td>Fine cream white fiber</td>
<td>99.0</td>
<td>0.5</td>
<td>5.0–7.5</td>
</tr>
<tr>
<td>Bamboo fiber</td>
<td>Fibrous white powder</td>
<td>99.0</td>
<td>0.3</td>
<td>4.0–7.0</td>
</tr>
<tr>
<td>Orange pulp fiber</td>
<td>Light yellow powder</td>
<td>68.2</td>
<td>2.65</td>
<td>5.0–7.0</td>
</tr>
<tr>
<td>Potatoes fiber</td>
<td>White powder</td>
<td>72.0</td>
<td>3.0</td>
<td>5.0–7.0</td>
</tr>
</tbody>
</table>

Methods of analyses

Titratable acidity (using 0.1 N NaOH for titration and phenolphthalein as an indicator) according ISO/TS 11869:2013. Total solids were measured by electronic moisture analyser (KERN MLS 50-3). The viscosity measurements were performed in triplicate at 18 °C in a rotational viscometer (Fungilab, Spain) with spindles R3 (juice) and R2 (yoghurt). Water holding capacity (WHC) was performed as described (Wu et al., 2013). 10 g of samples were centrifuged at 1800 g for 20 min at 25 °C. The sedimentable fraction was calculated by the ratio of the weight of sediment to the weight of the sample. All measurements were performed in triplicate. Arithmetic mean standard deviation was calculated for each sample.

Results and Discussion

Analyses of apple juice beverages

At first step apple juice was fortified with different kinds of fiber, as Sugar cane fiber, Bamboo fiber, Orange pulp fiber and Potatoes fiber. Sensory analysis indicated that only juice with Orange pulp fiber (Citri-Fi) and Potatoes fiber (Campus) was acceptable taste.

Quantitative solids evaluation of juice beverages samples (Table 2) indicated that apple juice with Citri-Fi fiber had highest amount of dry matter. The potato pulp fiber (Campus, 0.5%) reduced the dry matter in samples.

Table 2

<table>
<thead>
<tr>
<th>Samples</th>
<th>Dry matter, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citri-Fi M 40</td>
<td>11.92</td>
</tr>
<tr>
<td>Citri-Fi 200 FG</td>
<td>12.27</td>
</tr>
<tr>
<td>Campus</td>
<td>11.02</td>
</tr>
</tbody>
</table>

The apple juice acidity is expressed in the presence of malic acid. It is thought that Citri-Fi 200 FG fiber acids adsorbed more than another fiber used because this kind of fiber is natural mixture of citrus fiber and guar gum (Fig. 1).

Figure 1. Acidity of apple juice with different fiber

Sedimentation results are presented in Figure 2. The results showed that juice with potato fiber sedimentation was bigger than with citrus fibers.

Figure 2. Sedimentation of apple juice with different fiber

Viscosity flow dependency of used kind of fiber is showed on Fig. 3.
**Figure 3. Viscosity flow of apple juice with different fiber**

**Analisys of yoghurt**

Quantitative evaluation of solids of yoghurt samples (Table 3) indicated that yoghurt with sugar cane fiber had highest amount of dry matter. The orange pulp fiber 1% increased significantly the dry matter of samples compare to 0.5%.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Dry matter, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar cane 0.5%</td>
<td>13.47</td>
</tr>
<tr>
<td>Sugar cane 1%</td>
<td>13.5</td>
</tr>
<tr>
<td>Bamboo 0.5%</td>
<td>12.07</td>
</tr>
<tr>
<td>Bamboo 1%</td>
<td>12.75</td>
</tr>
<tr>
<td>CitriFi 0.5%</td>
<td>12.31</td>
</tr>
<tr>
<td>CitriFi 1%</td>
<td>13.24</td>
</tr>
</tbody>
</table>

The results of titratable acidity at the end of 3 weeks (21 days) exhibited the increasing in Figure 4. The highest influence on acidity was observed in yoghurts supplemented with sugar cane 0.5% and bamboo 0.5% fibers. The addition of fiber to 1% decreased variation of acidity.

As can be seen from Figures 6, 7, 8, the viscosity of yoghurt with different fiber exhibited shear-thinning flow behaviour. The viscosity of yoghurts with sugar cane (Figure 6) decreased during time. During cold storage the behaviour of viscosity didn’t changed.

The stability of yoghurt samples with fiber were characterized by sedimentation measurements as shown in Figure 5. The 1 day results showed that kind of fiber had no influence to sedimentable fraction. Otherwise, after 3 weeks the content of sedimentable fraction decreased. The experimental results were in agreement with increasing acidity when stability of yoghurt decreased. Therefore, the stability of samples with 1% of bamboo and Citri-Fi was constant.

**Figure 5. Sedimentation of yoghurts with different fiber**

**Figure 6. Viscosity flow of yoghurts with sugar cane fiber**

The same viscosity decreasing during storage was observed with Citri-Fi fiber (Figure 7). Results indicated that concentration of fiber had no influence. The viscosity results with bamboo fiber didn’t showed the significant differences (Figure 8). Moreover, yoghurts with sugar cane and bamboo fiber were less viscous at the end of storage. The Citri-Fi fiber helped yoghurt to keep structure more constant.

**Figure 7. Titratable acidity of yoghurts with different fiber**

**Table 3**

Total solids of yoghurt

<table>
<thead>
<tr>
<th>Samples</th>
<th>Dry matter, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar cane 0.5%</td>
<td>13.47</td>
</tr>
<tr>
<td>Sugar cane 1%</td>
<td>13.5</td>
</tr>
<tr>
<td>Bamboo 0.5%</td>
<td>12.07</td>
</tr>
<tr>
<td>Bamboo 1%</td>
<td>12.75</td>
</tr>
<tr>
<td>CitriFi 0.5%</td>
<td>12.31</td>
</tr>
<tr>
<td>CitriFi 1%</td>
<td>13.24</td>
</tr>
</tbody>
</table>
Figure 7. Viscosity flow of yoghurts with orange pulp fiber

Figure 8. Viscosity flow of yoghurts with bamboo fiber

Conclusions

Results showed that apple juice could be produced by using citrus and potatoes fiber. The essential physicochemical parameters of apple juice depending on fiber kinds remained essentially unchanged, but juice with Citri-Fi 200 FG fiber which guar gum adsorbed more malic acid.

Enriching yoghurt with bamboo and orange pulp fiber offered a scaffold that strengthened the yoghurt's structure, and increased stability during storage. The experimental results have demonstrated that as a potential stabilizer, the orange pulp fiber – Citri-Fi 100 has shown promise for the stabilising yoghurt system.

Acknowledgment

The authors wish to thank JSC Obuoliu namai (Kaunas, Lithuania) for the donation of apple juice and JSC Vijana (Kaunas, Lithuania) and Macorsus (Kaunas, Lithuania) for fruit by-products and for financial support.

References

DEVELOPING OF EDIBLE PACKAGING MATERIAL BASED ON PROTEIN FILM

Ivan Shatalov1, Alexandrina Shatalova2, Aleksandr Shleikin1

1Department of Food Technologies, Faculty of Chemistry and Molecular Biology, Institute of Refrigeration and Biotechnologies, ITMO University, Lomonosova Street 9, Saint-Peterburg, Russian Federation, e-mail: shleikin@yandex.ru
2Department of Food Technologies, Faculty of Technologies of Fish, Meat Products and Preserving by Cold, Institute of Refrigeration and Biotechnologies, ITMO University, Lomonosova Street 9, Saint-Peterburg, Russian Federation

Abstract

Nowadays due to the accelerated pace of industrial development in particular in food processing the necessity of the new kinds of packaging materials with desired functional properties (such as bacteriostatic activity for increasing the shelf life for raw materials and ready products) arises. However, the problem of using of non-degradable plastics in packaging that harms the environment remains unsolved. In this study we have attempted to develop an edible and biodegradable packaging material possessing activity against spoilage causing microorganisms of fruit and vegetable products.

Keywords: microbial transglutaminase, gelatin film, strength, elasticity, bacteriostatic activity.

Introduction

In world practice different film-forming materials used for food packaging which are produced as synthetic and biogenic composites. The leading trend of scientific developments in this direction is the creation of edible coatings.

The edible films can be obtained from protein, polysaccharide and lipid substances. Among them, the most attractive are the edible protein-based films. They have higher barrier properties than films produced from lipids and polysaccharides. However, poor stability of the protein films to water vapor and their low mechanical strength are limited their using in food packaging. Thus, modification of protein-based films must be aimed primarily at improving the mechanical strength and barrier properties of the packaging material with respect to moisture (Bourtoom, 2009).

Chemical modification helps to achieve increasing plasticity (Park et al., 2008). The most commonly used plasticizers include various polyols (glycerine), oligosaccharides and lipids (monoglycerides, phospholipids) that are destroying the hydrogen bonds between polymer chains, make structure more fluid, thereby increasing the elasticity. However, the barrier properties of the film are reducing (Hettiarachchy, Eswaranandam, 2005); besides these agents significantly increase the hydrophilicity of the material and as a result, increase its vapor permeability.

Successful attempts of increasing the elongation at break of the films were obtained when was applied formaldehyde and ethylene glycol (Wu, Zhang, 2001), however, due to the high toxicity of these compound, they can’t use in the food industry.

The most promising approach for modifying the structure of the protein components of the films is the use of enzymatic methods, and a special place among them is the use of microbial transglutaminase (mTG).

TG (protein-glutamine γ-glutaryl transferase, EC 2.3.2.13) – common in the nature enzyme involved in vital biological functions. mTG catalyzes an acyl-transfer reaction between the γ-carboxyamide group of peptide - bound glutamine residues (acyl donor) and various primary amines (acyl acceptor), including ε-amino group of lysine residues. This crosslinking may be both intra- and intermolecular that in the latter case leads to an increase in molecular weight protein molecules. The reaction proceeds according to the scheme:

\[
R_1-\text{Glu-CO-NH}_2+\text{H}_2\text{N-R}_2 \rightarrow R_1-\text{Glu-CO-NH-R}_2+\text{NH}_3
\]

Catalysis involving mTG led to various changes of films properties depending on the protein used but there was an increase barrier and mechanical properties in all cases. Thus, in the case of whey protein concentrate, soy isolates (SI) and their mixtures in various ratios were used, reduction in permeability to oxygen and water vapor were observed, but the elongation at break was increased under these conditions (Su, 2007). The vapor permeability decreased when mixture of gelatin and casein were used (Chambi, 2006). The vapor permeability decreased and the polymeric structure compressed when mixture of gelatin and sodium caseinate were used (Bruno, 2008). Films obtained from fish gelatin and treated by microbial TG had shown reduced transparency and reduced elongation at break, compressive structure and good barrier properties (Yi, 2006). In cases of film material comprising various composites by the enzyme treatment also leads to an improvement of some characteristics of the obtained materials. Thus, pectin – SI films prepared using mTG, show an increase in strength characteristics, but the elasticity of the films decreases (Mariniello et al., 2003). Processing transglutaminase caused an increase and decrease in the mechanical strength of the strain. Finally, it has been found that the effectiveness of the barrier to oxygen and carbon dioxide in an enzyme – linked films markedly improved, with decreased permeability to water. These data showed promising application of transglutaminase for the regulation of the mechanical properties and films permeability containing proteins (Di Pierro et al., 2006). The processing enzyme – ovalbumin chitosan films decreases their solubility in water and increases the mechanical strength, barrier properties against gases and water vapour at the same time, and also reduces the

298
amount of swelling (Di Pierro, 2007). Along with the development of biodegradable films, there has been actively working on giving them antibacterial properties using different agents: nisin (Rossi-Márquez et al., 2009), propylparaben (Chung et al., 2001), potassium sorbate (Flores et al., 2003), lysozyme (Buonocore et al., 2003).

The protein film can also function as a carrier of antimicrobial agents (Franssen et al., 2002). The ultimate goal of these modifications is to increase the retention and preservation of food raw materials and ready products. This work proposes to use as a packaging material with preservative properties of gelatin-based film, and its influence on the characteristics of the product is described below.

Materials and Methods

A commercial preparation of mTG Activa EB with an activity of 100 units (Adjinomoto Co), glycerol (Vector), porcine pig gelatin (LLC Norden), nisin (LLC Norden), potassium sorbate (LLC Norden) were used in the experiments.

Composition of films

In the study the composition of the film material containing gelatin and glycerine was used. There were some variants of films with different compositions at the previous stage. The film with the best organoleptic characteristics like transparency, strength, elasticity was chosen. The enzyme was applied in accordance with the recommendations of technology – supplier (0.01 g g⁻¹ protein). Because protein films are very fragile plastisizers are added to the composition for giving them elasticity. Glycerine was used as plastisizer in the study. Composition of analyzed material is shown in a Table 1.

Table 1

<table>
<thead>
<tr>
<th>Component</th>
<th>Test sample</th>
<th>Control sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Gelatin</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Glycerine</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>mTG, U</td>
<td>4.3 10⁻³</td>
<td>0</td>
</tr>
<tr>
<td>Potassium sorbate</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Nisin</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Water</td>
<td>to 100 mL</td>
<td></td>
</tr>
</tbody>
</table>

Preparation of film

Gelatin was dissolved in water at a temperature of 55–60 °C. After that glycerin was added to the solution of gelatin. The mixture was stirred and then cooled to 25 °C. Then mTG preparation was added, mixed well and placed in an incubator for 30 minutes at 37 °C. Then the predissolved preservative mixture was added to the film-forming solution. After that the volume of the mixture brought to 100 mL. The obtained solution (40 mL) is uniformly distributed over the surface coated with polyethylene (S=17×17 cm). These films were dried at room temperature and relative humidity of 50–60% within 18 hours.

Mechanical characteristics analysis

Before the test, the film thickness of the total area was measured at 10 points with accuracy of 1 micron. The mechanical characteristics study of the films, namely the tensile strength and elongation at break was performed on a tensile testing machine IR 5071-01S (LLC "Tochpribor Service") under standard conditions:

- distance between the clamps – 50±1 mm;
- sample size: width 15±1 mm, length – 100–150 mm;
- speed in relation to the moving clamp was 250 mm min⁻¹;
- measuring range from 0 to 10 kg (100 N ).

Tests were conducted in the dry state of the sample, the breaking strength was determined in the longitudinal direction of the sample. Breaking load (Qmax) and elongation at break (Lmax) was calculated using the following formulas:

\[ Q_{\text{max}} = \frac{F}{S}, \]

\[ L_{\text{max}} = \frac{l - l_0}{l_0}, \]

\[ X = \frac{n \times 10^m}{70.5}, \]

where:

- F – the applied load, S – section area of the sample
- \( l_0 \) – initial length of sample
- \( l \) – final length of sample
- \( m \) – number of microorganisms colonies on Petri dish
- \( n \) – amount of microorganisms colonies on Petri dish
- \( m = 4 \) in this study

All washes were carried out on three samples. After washout, the sample was removed from the cold chamber.

Shrinkage of berries analysis

Berries samples coated with film and control samples without it were stored under described in previous paragraph conditions. At regular times (0, 3, 4, 10, 12, 14 days) they were weighed in triplicate. Further data processing was carried using the formula:

\[ X = \frac{n \times 10^m}{70.5}, \]
\[
\% = \frac{m - m_i}{m} \tag{4}
\]

\(m\) – initial weigh, \(m_i\) – weight of berry at the day of measurement.

**Data processing**

All the data were processed in the program Microsoft Office Excel 2010 (Microsoft Corp.).

**Results and Discussion**

**Mechanical characteristics**

During the experiments, data were obtained on tensile strength and elongation at break of films of various compositions. The measurement results were compared, the changes in mechanical characteristics percentages shown in Figure 1.

The breaking strength increases along with a decrease in elasticity, which was determined by elongation at break, in all cases of application of the enzyme. In our opinion, these changes occur because of the formation of crosslinks between the polymer chains of the protein, causing their mobility in relation to each other within the polymer network to reduce, by this reason there were decreasing in elasticity and increasing in strength.

**Bacteriostatic activity**

Manufactures is known to prolong the shelf life of the product by the addition of various preservatives on the surface of the fruit, so before our experiments, all berries were thoroughly washed with warm water for half an hour. Then they were dried for a day at room temperature and constant winding then the experiment was started which results are shown in Table 2 and Figure 2.

Evaporation is one of the main reasons of weigh losses during storage. Some authors reported that mTG is capable to decrease water vapour permeability (Di Pierro, 2007), so we can suggest that the mTG-treated films can decrease weigh losses because of water evaporation.

As we can see the significant decrease in microorganisms quantity is observed in case of film application. The strong tendency of microphlora reduction is notable during storage for packaged samples, so we can conclude that the coating is capable to decrease and restrain bacteria growth.

**Shrinkage during storage**

The results obtained showed the decrease in weigh loss during the storage for samples coated in film. It can be concluded that the film could prevent shrinkage.

**Conclusions**

We have developed and investigated edible packaging material based on porcine gelatin with preservative properties. Because of using cross-linking agent – mirobial transglutaminase, it showed acceptable mechanical properties. At the same time, because of the preservative composition used based on potassium sorbate and nisin, it demonstrated antimicrobial action. Selected packaging material composition had no significant effect on the appearance of the packaged product, and, if desired, can be washed away from the surface of the sample.
References

Abstract

Each year the price of food products gradually increases, and some economists point out that to reduce the food prices, the food production increase must be managed. But even if some people suffer from hunger, others simply discard their food, so the possible food production increment can also increase wasted food amounts. One of the possible solutions to food product insufficiency is to cut down wasted food amounts. The main aim of this paper is to identify the non-price related factors that influence people to waste their food, look at food wasting matter from seller and buyer point of view, and also to identify the solutions to food wasting reduction. The results are based on research of theoretical guidelines and pilot research conducted in 2013, in which participated 610 respondents. Pilot research results shows that people are less concerned about reducing food wastage when it comes to environmental problems, but are the most motivated if they see a real opportunity to save money. So to motivate people waste less people need to be shown how their food wasting habit affect them economically.

Keywords: packaging, environment, storage, planning, labelling.

Introduction

The global population exceeded seven billion people during 2011 and is predicted to reach 9.3 billion by 2050, with a projected increased food demand of 50–70%. Against this backdrop of rising demand, 868 million people are chronically under-nourished, equating to one in eight people worldwide. At the same time, it is estimated that over one third of all food produced globally for human consumption goes to waste (Bond et al., 2013).

A food-wasting problem can be viewed from very different perspectives, not only as social problem that influence people relationship and values, but also as economical problem that directly or indirectly affects people’s incomes and in general can negatively affect the environment.

The aim of this review paper was to summarize the research conducted previously, identify non-price factors that affect food wasting at household level, explore the influence of non-price factors on selected respondents, and provide the evaluation of the problem by identifying the results of the pilot research.

Materials and Methods

Primary sources for theoretical discussion and pilot research survey shape identifying are scientific papers, monographs, fundamental documents that are closely related to the subject, which could be found in the scientific databases and as free sources on the Internet. Papers were selected by the search terms and by the provided references in the studies that were found.

The pilot research was conducted from May till September 2013. The survey was posted on the Internet, and had 48 questions in Latvian regardless peoples’ eating habits that influence food wasting. 610 respondents took part in the survey: 345 were women and 265 – men.

Age distribution:
- 18-29 years: 135 respondents or 22%
- 30-49 years: 243 respondents or 40%
- 50-69 years: 196 respondents or 32%
- 70 and more years: 36 respondents or 6%

Results and Discussion

Wasted food is defined as food that is discarded and not fully consumed (Princeton University Dictionary, 2006); it is closely related to attitudes and behaviours. Food gets ‘lost’ if it is affected by structural causes such as weak infrastructure, technological obsolescence, lack of refrigeration, etc. (Gustavsson et al., 2011). If the food loss problem in poor countries could be solved by investing money in infrastructure, processing and storage technologies and facilities that is mostly by investments then in rich countries to solve a food wasting problem, it is necessary to change people's attitude towards food, their habits and even laws.

The food waste concept, however, is not so strictly defined and it varies from research to research. Moreover, classification itself can be specific to a particular region or culture, and can be affected by the eating habits of the researchers. Thus, the classification not only includes the stage of food that gets discarded, but also it can include the interpretation of what is considered avoidable and unavoidable food waste. While, for example, Langley et al. (2010) consider all preparation by-products and residues of food preparation inedible and therefore unavoidable, researches that cooperate with Waste and Resources Action Programme use an additional subcategory of possibly avoidable food. Possibly avoidable food is considered edible and defined as the food and drink that some people eat and others do not (e.g. bread crusts), or that can be eaten when food is prepared in one way but not in another (e.g. potato skins)” (Household Food …, 2009). As for avoidable food, studies generally agree that wholly unused and partly consumed food would be avoidable, but the classification of the post-preparations and consumption residues differ.
The pilot research data shows that the bigger part of the average food waste makes a so-called unavoidable food waste, though an avoidable food waste still makes a significant part of the total waste. Research data also shows that with the income growth, the part of the food waste in the total waste also grows, both unavoidable and avoidable. That can also be explained with pickiness this people can afford. For example, when some part of the food seems less suitable for eating (in a case of an unavoidable food waste), and that is why it simply gets discarded. Or also can be explained with the assumption that it is easier for them to throw away suitable food when people just don’t want to eat it anymore, or have it cooked in a such a big amount, that it isn’t possible to eat it all, before it spoils (in a case of an avoidable food waste).

Economics researchers view a food-wasting problem manly from demand and supply perspective, evaluating what can more affect decrement of wasted food amounts – the increase of food demand or the increase of food supply (Rutten, 2013). Researching how food wasting reduction can affect both sellers/producers and consumers. Assuming that the decrease of wasted food amounts at consumption level can lead to the increase of consumption of the products that previously would be discarded, so basically people would eat more, or consumers would just buy fewer products, so in a way instead of just discarding their food they wouldn’t buy it at all, saving some money. The latter possibility would negatively affect sellers and producers of those products. So sellers aren’t motivated to decrease food wasting. Though if consumers demand for food products decrease, it does not mean that sellers cannot increase their incomes, they can always try to offer some different products that would be demanded by consumers. Because that, not buying certain food products, consumers would save money, so they have opportunity to buy something else, or even save some money up, so they can buy something more expensive, what previously they couldn’t afford.

In general, the food waste decrease can positively or neutral affect consumers, and neutral or negatively – producers / sellers. That is why for producers / sellers it is not beneficial to decrease their own wasted food amounts or that consumers get more cautious about their food waste habits and start to waste less. Because that for producers/sellers their incomes are proportional to the sold products. Thus, it is only possible to decrease wasted food amounts if consumers are informed about food wasting problem in general.

There are several different factors that can affect food thoughtless discarding, and those factors are closely connected to the people’s shopping habits, understanding of the labelling, financial and envelopment concern etc.

**Table 1**

<table>
<thead>
<tr>
<th>Non-Price Factors that Influence Consumers' Wasted Food Amounts</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Factors</strong></td>
</tr>
<tr>
<td>Unawareness of wasted food amounts</td>
</tr>
<tr>
<td>The way of storing</td>
</tr>
<tr>
<td>Packaging</td>
</tr>
<tr>
<td>Environmental concern</td>
</tr>
<tr>
<td>Financial benefits</td>
</tr>
<tr>
<td>To buy list and meal planning</td>
</tr>
<tr>
<td>Not understanding / being not able to read labelling of the product</td>
</tr>
</tbody>
</table>

Source: made by the author
No doubt, that the price can affect how much food can be bought, and therefore discarded in the end, but the price is not the only essential factor that affect food wasting.

One of the factors that affect food wasting is the unawareness of the wasted food amounts, and the main reason for that is that discarded food don’t stay stored in the house for too long, and if consumers compare bought and discarded food amounts, the discarded part seems insignificant (Jones, 2004). Therefore becoming more aware of the food products that get wasted, would help save up money and maybe also would stimulate people to plan their meals more truthfully.

To be able to find out how aware people in Latvia are about food wasting amounts in their household, the pilot research survey contained a question about how often people think about the cost of the food that in the end gets discarded. In addition, to be able to get a more demonstrative answer, this question was analysed by searching the correlations with the question about the average household income.

The results showed that in the households with the lower average incomes people much often think about how much their discarded food cost them, so the lower incomes are the more aware of the wasted food amounts people are. However, as it was stated in the Figure 1, people with higher incomes waste way more, so they are also the ones that think about the cost of the waste way less, but their contribution to the total food waste is the greatest. That is why it is important to make people with higher incomes much more aware of the food waste problems and consequences.

The way people store their food products is closely connected to the fact how long those products can stay fresh and valid for the consumption (Gustavsson et al., 2011). So right storing helps to use food more expedient for a longer time.

It is hard to explore all possible storing methods of individuals, for that it would be necessary to live together in one household with respondents. But it is possible to search the correlation between existing storing places in the household and the amount of possibly avoidable food waste in total waste. As it is shown in the Table 2, people waste way less 0–24% of all food if they have a proper storage place for it. And the more storing places respondents have, the less food they waste in the end.

Packaging not only prolongs the freshness and validity of the product, but also makes it more safe for a use, so bought food can be used longer, and in the best-case scenario – fully, not wasting much of it (Manalili et al., 2011).

### Table 2

| Available places of storage and influence on wasted food amounts | Possibly avoidable food waste of all waste, % |
|---|---|---|---|---|
| **0–24** | **25–49** | **50–74** | **75–100** |
| A refrigerator with a freezer | Count | 517 | 35 | 12 | 3 |
| | % of Total | 85.8 | 5.7 | 2.0 | 0.5 |
| A refrigerator without a freezer | Count | 16 | 0 | 0 | 1 |
| | % of Total | 2.6 | 0.0 | 0.0 | 0.2 |
| More than one refrigerator with a freezer | Count | 35 | 3 | 0 | 0 |
| | % of Total | 5.7 | 0.5 | 0.0 | 0.0 |
| A freezer | Count | 113 | 6 | 0 | 0 |
| | % of Total | 18.5 | 1.0 | 0.0 | 0.0 |
| A storeroom | Count | 175 | 14 | 6 | 1 |
| | % of Total | 28.7 | 2.3 | 1.0 | 0.2 |
| A cellar | Count | 266 | 16 | 6 | 0 |
| | % of Total | 43.6 | 2.6 | 1.0 | 0.0 |
| Total | Count | 557 | 37 | 12 | 4 |
| | % of Total | 91.3 | 6.1 | 2.0 | 0.7 |

### Table 3

<table>
<thead>
<tr>
<th>Things customers pay attention when buying food</th>
<th>Responses</th>
<th>Percent of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Price</td>
<td>549</td>
<td>31.4</td>
</tr>
<tr>
<td>Ingredient list</td>
<td>294</td>
<td>16.8</td>
</tr>
<tr>
<td>Product's expiration date</td>
<td>471</td>
<td>26.9</td>
</tr>
<tr>
<td>Packaging</td>
<td>106</td>
<td>6.1</td>
</tr>
<tr>
<td>Product's volume or weight</td>
<td>330</td>
<td>18.9</td>
</tr>
</tbody>
</table>
The pilot research results show that respondents pay attention to the packaging way less, than to the price and product’s expiration date. Even if food validity is closely connected to the packaging.

But when it comes to the environment concerns Baker et al. (2009) came to concussion that it motivates to think about how to reduce food discards way less than the possibility to save some money. But people take it positively, that food wasting reduction and money saving can also help to positively impact environment.

The pilot research results showed similar situation as Baker et al. (2009) described, respondents do not care or seldom care about environment problems that are caused by food wasting.

People admit that planning meals and listing products that need to be bought can help reduce food wasting; because that can help with not buying unneeded products spontaneously. But research also show that no matter that people want to reduce wasted food amounts and eve plan what to buy, for them it is still hard not to make impulsive buys. Sometimes people also just make up their minds and do not want to eat anymore something that they previously planned and have bought food for (Stefan et al., 2012).

So in a way if the planning has a positive impact on food waste reduction, than the shopping routine has an opposite impact. Meaning that no matter how good a person previously thought about that to buy and what to do with bought food, during shopping it still is hard not to buy food, that wasn’t planned, and that this action in the end won’t be a reason for some food wasting (Stefan et al., 2012).

Gunders (2012) came to conclusion that main part of the consumers does not really plan what they are going to eat. Food gets impulsively bought (sometimes those action influence commercials, or sales, or the wants/needs of the person at that time), but then food gets stored and unused, sometimes food gets bought in such a big amount that it isn’t even possible to consume all of it before the expiration day or before it actually spoils. Researcher explains the buy of big amounts of food products with the policy sellers/manufacturers have, when it is more beneficial, because that calculating per kg, it is more beneficial, because that per kg that product is more cheaper.

The pilot research shows that planning does help with reducing unavoidable and also avoidable food waste. However, there does not exist a significant difference between values. That probably also can be explained by the conclusions Gunders (2012) came up in his research, that even if Latvian respondents plan their meals beforehand, for them it is still hard not to make impulsive buys.

Food labelling can be very misleading, people can buy food that contains ingredients that can cause allergic reactions, so not knowingly a person can buy it, and, if a product consumed fully, a person can have health problems, but if a product wasn’t fully consumer, it most likely will be discarded, so in the end – wasted. Food labelling also can mislead people in to thinking that the product is unsuitable for the consumption, assuming that expiration date shows the exact day when product must be discarded obligatory (Manalili et al., 2011).

It is hard to determine if respondents gets mislead by the labelling, because that most of the time (not having a bad allergic reaction, or product having no bad smell or taste) a person does not even understand that wrong/unsuited food was bought.

So it was important to identify answers to the question about if people deliberately buy products, whose validity has almost ended, and correlate those answers with answer to the question how people determine if the food is still valid for the consumption.

Those respondents that buy food whose expiration date is very close, more likely will judge the picked food suitability for the consumption not by date, but by the smell of the product.
Food labelling and respondents’ food validity determination methods

<table>
<thead>
<tr>
<th>How do you determine if food is valid for the consumption</th>
<th>Do you buy food whose validity will end soon?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Count</td>
<td>never</td>
</tr>
<tr>
<td>By the expiration date</td>
<td>Count</td>
</tr>
<tr>
<td>% within row</td>
<td>17.5</td>
</tr>
<tr>
<td>% within column</td>
<td>32.1</td>
</tr>
<tr>
<td>By the look of the product</td>
<td>Count</td>
</tr>
<tr>
<td>% within row</td>
<td>16</td>
</tr>
<tr>
<td>% within column</td>
<td>24.9</td>
</tr>
<tr>
<td>By the smell of the product?</td>
<td>Count</td>
</tr>
<tr>
<td>% within row</td>
<td>14.1</td>
</tr>
<tr>
<td>% within column</td>
<td>23.7</td>
</tr>
<tr>
<td>By the taste of the product</td>
<td>Count</td>
</tr>
<tr>
<td>% within row</td>
<td>16.4</td>
</tr>
<tr>
<td>% within column</td>
<td>19.3</td>
</tr>
</tbody>
</table>

Those people, whose food is still valid judged by the date written on the packaging, decide if food is valid for the consumption most often by its’ taste.

Conclusions

1. Food thoughtless discarding is affected by different factors, and even if the price of the food plays a very significant role in the future of the bought food, it is not the only factor that affect unavoidable and avoidable food waste.
2. Unawareness, storing, the packaging of the food, the environmental concern, financial benefits, planning and the labelling – all this non-price factors can have both positive and negative effect on food wasting.
3. People in Latvia have similar food wasting tendencies as foreigner researchers have observed during their own research.
4. In order for Latvian people to understand the seriousness of the food wasting problem in the world, people must be more often informed about food wasting problems and consequences. Not only on global scale, but also on personal level – how people’s actions affect them financially, because that financial factor is the most effective lever that can make people waste food less.

References

FOODBALT 2014

CURRENT EATING PATTERNS AND LIFE STYLE ON THE HEALTH OF NAMIK KEMAL UNIVERSITY STUDENTS

Emine Yilmaz, Yasemin Oraman, Gokhan Unakitan, Burcin Basaran

Department of Agricultural Economics, Agricultural Faculty, NKU, Tekirdag, Turkey, e-mail: emineyilmaz@nku.edu.tr

Abstract

The aim of this study to determine the students' current eating patterns and life style on the health. A self-reported questionnaire was administered to 618 students. Students from Namik Kemal University in Turkey participated in this study. Chi-square analyses were conducted for non-parametric variables. Descriptive statistics of some of the results obtained from the survey study are as follows: 39.8% of the students smokes, 55.3% of them come from the city, which 65.5% stay at a flat with their friends. It was reported that 38.2% of them have breakfast regularly, 45.1% have lunch regularly, and 79.6% have lunch regularly. In order to test whether or not there is any difference between the regular consumption of breakfast, lunch and dinner according to the students’ gender, chi-square test was made. In chi-square test made at p<0.05 level, it was discovered that there is difference in their consumption between the regular breakfast and lunch according to their gender. It was recognized that male students consume dinner more regularly. The most attractive foods are meat dishes, vegetable dishes, and meat pasty.

Keywords: eating pattern, life style on the health, student.

Introduction

It is obligatory for a living organism to feed itself in order to maintain its life (Çalisır et al., 2005). Nutrition is to use and take most economically the food which provides sufficiently each of energy and nutrition components that are necessary for growing, development and living healthily and efficiently for a long time without making it lose its nutrition value and destruct health (Tanrı et al., 2001). It is put forward scientifically that growing and development are prevented and that health is harmed when any of these components is taken insufficiently or excessively (Baysal, 1993). However, it must not be forgotten that nutrition is a sociological and psychological action as well as a physiological one (Çalisır et al., 2005). To be healthy in the mind and body and retain health are possible with sufficient and balanced nutrition (Tanrı et al., 2001).

Although nutrition is significant for each section of the society, it has a distinct significance for the university youth. Most of the students studying at university have to live away from their family environment for the first time in their lives. Before university, the nutrition habits of student continue as the family life requires, but the differentiating life with university can change the nutrition habits of the student. The changing nutrition attitudes not only concern the mental and physical situation of the student but also are able to affect his school performance. Therefore, determining the nutrition information and habits which the university students have and developing advices suitable for the situation are very important (Erten, 2006).

A considerable number of high education students study away from their families, and this situation causes difficulties in their nutrition, housing, school expenditures and health problems. The studies regarding the nutrition habits of the young at university report that very serious problems are lived in this period, that students usually do not care for meals, that they neglect meals, especially breakfast, that they eat such nutrients as bagel and tea and that satiating hunger and eating are accepted as identical among the young (Yaman, Yabancı, 2006; Karaoğlu et al., 2005). Sufficient and balanced nutrition carries significance on the grounds that it affects people’s health and successes and makes the following generations gain a good nutrition habit. This study planned from that was planned and conducted in order to determine the students’ information levels of nutrition and their nutrition habits at Namik Kemal University.

Materials and Methods

This research was conducted to determine the students' current eating patterns and life style on the health of the students surveyed. A self-reported questionnaire was administered to 618 students. Students from Namik Kemal University in Turkey participated in this study. The Questionnaire consisted of five parts and first part is about some personal information about students and their body composition. The second part contains questions that measure students’ eating behaviour and its relation to nutrition knowledge. The third part includes some questions about student’s attitudes toward nutrition habits. The forth part poses some questions about choosing the foods and the last part collects student’s recommendations. The collected data was analysed means, standard deviation and percent were calculated. In addition, this result supported by chi-square test for non-parametric variables.

Results and Discussion

It was determined that 38.3% of the students participating in the research were female, and 61.7% were male; 87.1% were between 18–21 ages, and 20.0% were between 22–25 while 1.9% were over 26; their age average was 20.5. It was found out that 55.3% of them come from the city which 65.5% stay at a flat with their friends which mothers of 48.5% graduated from primary school while fathers of 34.4% graduated from primary school (Table 1).

Keywords: eating pattern, life style on the health, student.
According to Leigh (1983) smoking is a habit which affects health negatively. While a study conducted on students at Cukurova University reveals that smoking rate of girls was 39.0%, another study conducted on students at Ankara University reports that 27.9% of the students smoke 17–20 cigarettes a day (Yaman, Yabancı, 2006). In this study, 39.8% of the students smoke. The proportion of the students smoking 1–5 cigarettes a day is 12.2% while that of the students smoking 16 and more cigarettes a day is 40.2%.

As nutrition education can be achieved with an individual’s training himself in his own conditions, it can also be provided for individuals by means of formal and common education institutions. In this study, 22.3% of the students stated that they took lessons regarding nutrition whereas 18.8% expressed that they participated in the meetings such as conferences and panel (Table 1). Mazıcıoğlu and Öztürk (2003) reported that 47.2% of the students took education regarding nutrition and that 27.7% of those taking education participated in a conference and education on nutrition outside the lesson while Ertın (2006) in his study indicated that 27.7% of the students took education related with nutrition and that 98.5% of them took this education at school.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Female %</th>
<th>Male %</th>
<th>Total %</th>
<th>X²/p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breakfast</td>
<td>209 (44.3)</td>
<td>336 (34.6)</td>
<td>545 (38.2)</td>
<td>X²=3.399 p&gt;0.05</td>
</tr>
<tr>
<td>Lunch</td>
<td>117 (35.6)</td>
<td>313 (51.2)</td>
<td>430 (45.1)</td>
<td>X²=12.953 p&lt;0.05</td>
</tr>
<tr>
<td>Dinner</td>
<td>159 (79.7)</td>
<td>231 (79.6)</td>
<td>390 (79.2)</td>
<td>X²=0.018 p&gt;0.05</td>
</tr>
</tbody>
</table>

In order to test whether or not there is any difference between the regular consumption of breakfast, lunch and dinner according to the students’ gender, chi-square test was made. In chi-square test made at p<0.05 level, it was discovered that there is difference in their consumption between the regular breakfast and lunch according to their gender. It was recognized that male students consume dinner more regularly.

The information about the students’ meal consumption is given in Table 3. It was reported that 38.2% of them have breakfast regularly, 45.1% have lunch regularly, and 79.6% have dinner regularly. It was found out the food consumed most at breakfast is pastry, toast, bagel (43%), standard breakfast cheese, onion, jam, etc. (54.9%), cornflex+ milk (2.0%), and those consuming only tea or coffee is 4.8%.

It was informed in another study conducted in Ankara that the most preferred food by university students is cheese-onion (67.4%) and bagel (53.7%) (Güllec et al., 2008).

In our study, it was mentioned that breakfast is omitted most while dinner is neglected least. In order to take the energy and food constituents at the suggested level in the youth age, the consumption of the main meal and snack must be approached with care. The number of meals is important in sufficient and balanced nutrition. While neglecting meals, especially breakfast, affects school success negatively in children and young people, it also paves the way for insufficient and unbalanced nutrition (Rampersaud et al., 2005; Dimiglio, 2000).

In a study which Tumerdem et al. conducted on the students studying in different faculties of three universities in Istanbul, they discovered that breakfast and lunch are not taken regularly, and hunger is satiated...
with hurry while dinners are prepared more carefully than other meals (Tumerdem et al., 1985). In a study which Arslan et al. (1994) conducted on young people of high education, it was found out that the most neglected meal is breakfast with 31.5%. Saygun (1987) determined the fact that breakfast is most often neglected among other meals. Isıksologlu (1986) and Ozpınar (2002) discovered that the least neglected meal is dinner. In a study which Nnanyelugo and Okeke (1987) performed on the university students in Nigeria, it was determined that the most neglected meal is breakfast by the proportion of 73%. Often, neglecting breakfast can spring from the difficulty of preparing breakfast and worry to be late for school. However, it is reported that breakfast has a very important role in beginning day eagerly, maintaining the activity suitably and preserving the level of sugar blood (Erten, 2006).

The students participating in the survey were asked what kinds of food attract their attention, and Table 3 was made up. The most attractive foods are meat dishes (4.71), vegetable dishes (4.63), and meat pasty (4.55). The least attractive foods are frozen food (3.37), canned food (3.40), eggs (3.68), fast food (3.86), and pizza (3.86) (Table 4).

In recent years, fast-food consumption has been increasing with the increasing importance of time and the effect of the consumption habits imported from developed countries on the university youth. The fact that the young population demands much for this area has increased the fast-food sector in cities especially where universities exist. When the conducted researches are examined, it is seen that most of the students have fast-food consumption habit most (because it is the most practical one). As the young people consume much fast-food, home cooking attracts the students’ attention more.

Conclusions

In this study in which the information and habits of university students regarding nutrition were evaluated, it was discovered that the students neglect meals and that the most neglected meal is breakfast (61.8%). 22.3% of the students stated that they took education concerning nutrition, and 18.8% stated that they participated in such meetings as conferences and panels. The foods mainly attracting the students’ attention are meat dishes, vegetable dishes and meat pasty. The foods attracting the students’ attention least are frozen food, canned food, eggs, fast-food and pizza, and we can say that the reason for this is that they consume these foods more.

What is important is that necessary precautions should be taken before the students’ consumption habits become permanent. Of these precautions, making up healthy nutrition habits and enabling healthy food to be reached come first. Therefore, individuals should be made to adopt the habit of consuming main meals regularly from their infancy and childhood age. It is though that the fact that the students adopt the right consumption habits is important in terms of protecting their health in their following periods of their lives (Rampersaud et al., 2005). For this reason solving the incompetence in nutrition information and habits with the nutrition education given permanently and effectively, organizing short-term conferences, panels and conversations on nutrition issues at universities at regular intervals will be useful in solving the problems concerning nutrition of the students.

References


SHORT COMUNICATION

OPTIMIZATION OF MANUFACTURING TECHNOLOGY OF SOFT CHEESE

Ruta Budreckiene, Agne Struzeckiene

Department of Biochemistry, Veterinary Academy, Lithuanian University of Health Sciences, Tilzes str. 18, Kaunas, Lithuania, e-mail: rutbudr@lva.lt

Abstract

The aim of this work was optimization of production processes and determination of the goals settings of the optimal values in production of investigatory soft cheese. Consequently, fat and protein ratio of the prepared pasteurized milk mixture for soft cheese production, and their influence on the fat content in dry matter of final product was determined, moisture of the coagulum and the final product established and the coagulum moisture content and coagulum drying effect on the duration of the final product moisture investigated.

It was established that the largest fat content in dry matter of soft cheese (52.21%) is by the fat/protein ratio of – 1.21, and the lowest (41.69%) – by the ratio of 0.83. Optimal pasteurized mixture of milk fat/protein ratio for soft cheese is from 0.830 to 0.962. The ideal fat/protein ratio for given soft cheese – 0.89. The lowest moisture content of the final product (51.9±0.08%) was obtained by the coagulum moisture at 76.1±0.07%, and the highest – 55.0±0.09%, when coagulum moisture was 71.4±0.13%. The optimal coagulum moisture range, conforming to corporate standards for the final product moisture soft cheese is from 69.01 to 75.55%. The longest drying time of the coagulum (35 min) corresponds to the minimum moisture content of soft cheese – 51.43±0.492%. The maximum moisture content of soft cheese 54.87±0.38% was obtained, when the coagulum drying time was 30 min. This period of time is also optimal drying time for coagulum of investigatory soft cheese.

Keywords: milk, soft cheese, coagulum.

Introduction

Production of cheese has started in the year 8000 BC, according to the various sources. Cheese is ripened or unripened, soft or semi-hard, hard or very hard product (which may have a coating or shell), and the protein (casein) ratio of whey should not exceed the amounts in milk (Sekmokien et al., 2008).

The quality of produced cheese acts the composition, quality and activity of the leaven of the milk used in tanning for the cheese production, decisive. Cultures micro flora catalyzes the enzymatic milk clotting process, stimulates enzymatic curd syneresis and provides unfavorable conditions for the secondary micro flora. Cultures micro flora changes the main milk components (lactose, proteins, lipids) in the compounds, leading to cheese flavor, aroma, nutritional and biological value. Compounds, secreted of cultures micro flora, in particular CO₂, form the porosity and consistency of cheese (Rupsiene, 2003).

Soft cheese is characterized by the fact that there is a considerable amount of moisture, which causes a variety of problems if quantity of humidity is too high. Excessive moisture can cause unwanted microorganisms appearance and reproduction, therefore, in order to avoid of this, term of realization has to be short what is useless for the company in respect of sales. Also product loses marketable appearance, its organoleptic properties becomes lower (Gudonis, 2012; Masteikienie, 2006).

This investigation was focused on the one kind of dairy product – soft cheese. All the production from entering the establishment of milk to the finished product is analyzed, with the aim of optimization of production processes and determination of the goals settings of the optimal values in production of investigatory soft cheese.

Materials and Methods

This research was carried out in the Department of Biochemistry of LHSU and in the company “Modest”. The cheese-making technology. Milk for the production of cheese is normalized in fat and protein relation. To obtain the corresponding final product oiliness, the appropriate relationship of fat and protein must be used. Normalized mixture is heated to 35–45 °C and cleaned. Finally, in order to obtain the required normalized fat milk and a skim milk of minimum fat content, it must be separated. The following sequence is: filling of production machines, heating of milk, tanning and clotting of milk mixture, curd processing, cheese making, pressing, and the salting. Finally, the cheese is ripened and realized (TI-10-PL-3).

Milk analyzer Lactoscope C4 + (Holland) was used to detection of milk fat, protein, solids and freezing point. This spectrophotometer analyzes the milk and milk products composition using infrared technology. The moisture content was determined using a moisture analyzer KERN MLB-N.

In the statistical analysis of the data arithmetic averages of groups were calculated, the errors were estimated and statistical significance of intergroup was established (ANOVA, Duncan multiple comparisons test). The correlation coefficients were counted and regression analysis (linear and square studied addiction) was conducted for evaluation of relations between the characteristics of subjects. Calculations were made with the computer program Microsoft Excel 2007. The study is considered reliable at p<0.05, unreliable – if p>0.05.
Results
Soft cheeses are moist, and it causes many problems for the product. Undesirable microorganisms may appear due the high moisture content inside the cheese. In order to avoid this, a short term of product sales is recommended. Product loses marketable appearance, decreases its organoleptic characteristics, at the same. Consistency of the product becomes fragile; the product is crumbling, it is impossible to dice it nicely. This soft cheese is cut by hand and evacuated in the company; these conditions causes large yield losses because of large amounts of scrap and are not suitable for sale.

It was very important to optimize the production technology of soft cheese so as to control the moisture and get the best possible texture of the product, to minimize financial losses of company and to satisfy the buyer with the result. In order to achieve these targets it was necessary to look over the cheese-making technology and perform tests, focusing on normalization of pasteurized milk mixture in respect of fat/protein ratio, estimation of grain moisture and grain drying time impact on the quality of the product and evaluation and determination of the optimum values of these parameters in the production process.

Test of the pasteurized milk mixture fat / protein ratio influence on the final product. The milk supplied to the company is usually too rich in fat and has too high protein content. Dairy products derived from such non-standardized milk are very creamy and low consistency – soiled, without form and excessive fat content can lead to extraneous taste, also. Therefore, in the milk industry in order to get cheese of standard fat and protein content milk is normalized by normalization instructions with skim milk, buttermilk or sour cream. This is the most important step, which determines the chemical parameters of the forthcoming final product.

Rations of fat and protein in pasteurized milk mixture were presented in Figure 1.

The fat content on a dry basis of the final product increases with the increase of the fat / protein ratio. Since the linear equation describes the dependence statistically reliable, we can predict the fat / protein ratio limits, hoping to get optimal fat content on a dry basis of soft cheese. To obtain the final product within the fat content of the dry basis of 45±2%, the fat / protein ratio should range from 0.830 to 0.962.

Influence of grain moisture on the moisture of final product. Cheese grains obtained during the manufacturing process distinguish the whey and forms a peel. If the peel arises very quickly, the whey fails to stand out from grains, so in grains, and thus the in mass of cheese, could increase the number of micro-organisms activity. Otherwise, when the peel arises very slowly too much whey is eliminated from the grain and the product becomes very dry and crisp. His consistency is crumbling.

During the technological process, when curd cutting, mixing and drying of grains were executed, the samples from the production machines were selected. The moisture contents in the milk samples were measured using a moisture analyzer KERN. In the Table 1 the average values of moisture in grains and in soft cheese are presented.

From the data presented in Table 1 it is evident that there is no linear relationship between the moisture of soft cheese and grains: initially, with the increase of grain moisture the moisture content of soft cheese is also growing, but later, after the optimum point is reached, soft cheese moisture begins decline, although grains moisture continues to increase. The maximum value of the final product moisture 55.0±0.09% is reached when the grains moisture is 71.4±0.13%.

Figure 1. Dependence of the fat in a dry basis of soft cheese on fat/protein ratio of pasteurized milk mixture

The data showed that the increase of fat / protein ratio leads to the growing of the fat in a dry basis of soft cheese, almost evenly. The highest it is in fat / protein ratio of 1.21, and the lowest at 0.83.

The graphical regression analysis of the interdependence of these properties is presented in Figure 2. It was set very strong linear correlation (r=0.96, p<0.001) among the fat / protein ratio and the fat in a dry basis of soft cheese.

Figure 2. The interdependence regression analysis of fat/protein ratio and fat in the dry basis of soft cheese

The data showed that the increase of fat / protein ratio leads to the growing of the fat in a dry basis of soft cheese, almost evenly. The highest it is in fat / protein ratio of 1.21, and the lowest at 0.83.

The graphical regression analysis of the interdependence of these properties is presented in Figure 2. It was set very strong linear correlation (r=0.96, p<0.001) among the fat / protein ratio and the fat in a dry basis of soft cheese.
The influence of soft cheese moisture on the grains drying time. 

Table 2

<table>
<thead>
<tr>
<th>Test No.1</th>
<th>Test No.2</th>
<th>Test No.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grain drying time</td>
<td>25 min</td>
<td>30 min</td>
</tr>
<tr>
<td>Soft cheese moisture, %</td>
<td>53.4±0.4</td>
<td>54.87±0.38</td>
</tr>
<tr>
<td>a, b indicates a statistically significant differences at p&lt;0.05. C, D indicates a statistically significant differences at p&lt;0.001.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The average values of moisture in grains and soft cheese, %

<table>
<thead>
<tr>
<th>Production No.</th>
<th>In grains</th>
<th>In soft cheese</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>67.8±0.11</td>
<td>52.3±0.06</td>
</tr>
<tr>
<td>2</td>
<td>68.7±0.11</td>
<td>52.4±0.07</td>
</tr>
<tr>
<td>3</td>
<td>69.6±0.22</td>
<td>52.4±0.11</td>
</tr>
<tr>
<td>4</td>
<td>69.9±0.09</td>
<td>52.7±0.08</td>
</tr>
<tr>
<td>5</td>
<td>70.1±0.07</td>
<td>53.1±0.07</td>
</tr>
<tr>
<td>6</td>
<td>70.2±0.09</td>
<td>53.8±0.06</td>
</tr>
<tr>
<td>7</td>
<td>70.9±0.07</td>
<td>54.5±0.06</td>
</tr>
<tr>
<td>8</td>
<td>71.4±0.13</td>
<td>55.0±0.09</td>
</tr>
<tr>
<td>9</td>
<td>72.4±0.09</td>
<td>54.6±0.05</td>
</tr>
<tr>
<td>10</td>
<td>73.5±0.09</td>
<td>54.2±0.04</td>
</tr>
<tr>
<td>11</td>
<td>74.1±0.05</td>
<td>54.0±0.03</td>
</tr>
<tr>
<td>12</td>
<td>75.0±0.06</td>
<td>53.3±0.03</td>
</tr>
<tr>
<td>13</td>
<td>75.2±0.06</td>
<td>53.0±0.05</td>
</tr>
<tr>
<td>14</td>
<td>75.8±0.04</td>
<td>52.6±0.03</td>
</tr>
<tr>
<td>15</td>
<td>76.1±0.07</td>
<td>51.9±0.08</td>
</tr>
</tbody>
</table>

The dependence of soft cheese moisture on the grains moisture described using the quadratic equation. Statistical significance of addiction was evaluated calculating the Fisher criterion (F=3291.2, p<0.001 (p=4.01×10^-54)). Equation can be statistically significant (p<0.001) predicted that the moisture of the final product will vary within the permitted limits (53–55%), when grain moisture content will vary in the range from 69.01 to 75.55%, according to the dependence. The influence of the moisture of soft cheese on grains drying time. The grains drying time is particularly important process that determines the appearance of the product. It depends on the season of the year and from the composition of the milk changes. The grains drying time is determined by grains moisture, which is important in order to get a good consistency of the final product. The data presented in Table 2 shows influence of soft cheese moisture on the grains drying time.

The graphical dependence of the final product moisture on the grain moisture is given in Figure 3.

Figure 3. The soft cheese moisture dependence on the grains moisture.

The importance of milk composition on the final product is very important in milk industry. Too high fat content in the mixture can damage the sensory properties of produced cheese and the product could be too soft, can not have its inherent consistency, moisture content of product will become too high and it could start multiplying of various bacteria. Too low level of fat content in the milk mixture will make the product lean, dry, it could become free-flowing and crumbling. According to company standard IST 121313693-01:2009, the ideal ratio of fat / protein in a milk mixture is 0.89, during our experiment (Fig.1). The grains size of the cheese is very important for the moisture content of cheese mass. The whey will excrete much faster from the smaller grains than from the larger. Together with the whey and dissolved substances, releases colloidal and emulsion components of milk. Because of the distinguishing of whey from the cheese grains, a peel forms on the surface. If the peel arises very quickly, the whey fails to stand out from grains, so in grains, and thus the in mass of cheese, could increase the number of micro-organisms activity. So, it is very important to determine appropriate moisture of grains to obtain standards-compliant product. Moisture content of final product in SC Modest enterprise standard for soft cheese is defined 53–55% (IST 121313693-01:2009). When the moisture of
grains is 67.818%, the moisture of soft cheese – 52.268% (does not meet to the indicator defined by standard of the company). When grains moisture is 71.418%, moisture of soft cheese – reaches 55.038% (optimal variant of declared parameters), while the moisture of grains was 76.148%, soft cheese moisture was just 51.898% (does not meet to the indicator defined by standard of the company). The optimum range for grains moisture is from 69.01 to 75.55%.

All the studies and analysis of the results were based on our own assumptions and conclusions, as neither Lithuania’s nor foreign authors on a similar theme was unable to find and compare.

**Conclusions**

1. The largest fat content in dry matter of soft cheese (52.21%) is by the fat / protein ratio of – 1.21, and the lowest (41.69%) – by the ratio of 0.83. Optimal pasteurized mixture of milk fat / protein ratio for soft cheese is from 0.830 to 0.962. The ideal fat / protein ratio for given soft cheese – 0.89.

2. The lowest moisture content of product soft cheese (51.9±0.08%) was obtained by the coagulum moisture at 76.1±0.07%, and the highest – 55.0±0.09%, when coagulum moisture was 71.4±0.13%. The optimal coagulum moisture range, conforming to corporate standards for the final product moisture soft cheese is from 69.01 to 75.55%.

3. The longest drying time of the coagulum (35 min) corresponds to the minimum moisture content of product soft cheese – 51.438±0.492%. The maximum moisture content of soft cheese 54.87±0.381% was obtained, when the coagulum drying time was 30 min. This period of time is also optimal drying time for coagulum of investigatory soft cheese.

**References**

SHORT COMMUNICATION

PIKE-PERCH FARMING IN RECIRCULATING AQUACULTURE SYSTEMS (RAS) IN THE KALININGRAD REGION

Dmitry Pyanov, Artem Delmukhametov, Evgeny Khrustalev

Department of Aquaculture, Faculty of Bioresources and Nature Management, Kaliningrad State Technical University, 1 Sovietsky prospect, Kaliningrad, Russian Federation, e-mail: dmpjanov@gmail.com

Abstract

Pike-perch (Sander Lucioperca L.) is considered as one of the most valuable food fishes native to Europe. Low fat content (1–2%), highly assimilable protein and a delicate flavour makes pike-perch meat highly valued by dieticians. Currently, pike-perch available on the market are usually caught by fishers, mainly from Eastern Europe. However, breeding technology is still low, only a few countries (such as Denmark and the Netherlands) have established production. According to predictions of increasing consumption and environmental degradation it’ll be necessary to develop reliable technology of pike-perch reproduction as a potential for aquaculture development. Nowadays, in the EU is actively developing aquaculture in fully “recirculating” systems where water is largely reused. It is known as Recirculating Aquaculture Systems (RAS). In the EU, only the fish which is reared in the RAS may be considered as an eco-friendly product. And food safety concerns are led to the fact that some consumers are willing to pay a premium prices for safe and eco-friendly products, making such cultivation technology more profitable. The objective of this article is to provide an overview of the current status of pike-perch farming in the Russian Federation with a focus on quality of fish, which is produced in environmentally friendly way by using the RAS technology. We researched the growth features of various pike-perch generations reared experimentally in a local fish hatchery and established the possibility of introducing this species to the market in the Kaliningrad Region.

Keywords: pike-perch, RAS, weight gain.

Introduction

Nowadays, aquaculture is a highly dynamic sector of agriculture. This fact can be illustrated by data of American analytic company “Transparency Market Research” report about aquaculture market development. According to company prediction, aquaculture market production in 2019 will be valued at US$ 195.13 billion. Towards the end of 2015 the volume of aquaculture production presumably to exceed the amount of fish caught, and fishery will cease to be the main source of fish and seafood supplies for human consumption (Transparency Market Research, 2013). The strong environmental restrictions to minimize pollution from hatcheries and aquaculture plants in Northern European countries have sparked a rapid technological development of recirculating aquaculture systems (RAS). The RAS enables to create optimal conditions for farmed fish at all stages of the production process and control the health of farmed fish. The highest possible outcome of fish products per unit area is common to the system in particular. That is a compromise with regard to intensive production and environmental sustainability at the same time (Bregnballe, 2010). Pike-perch (Sander Lucioperca L.) is a new species for cultivation in the RAS. This fish is characterized by rapid growth, early maturation and high breeding performance, complemented by excellent taste of meat (Хрусталев, Дельмухаметов, 2010). Thanks to its low fat content (usually 1–2%) and highly assimilable protein, pike-perch meat is highly valued by dieticians and suitable for baby food production (Мухатова, Гайдыроиська, 2010). The proportion of the unsaturated fatty acids is high and the proportion of the omega-3/omega-6 (n-3/n-6) fatty acids (3:1:4:1) might help to develop the ideal level in the human body (Molnár et al., 2009). Pike-perch is usually sold frozen as gutted whole fish, fillets with skin or skinned fillets. The fillets are usually sold in the following weight categories: 120–170 g, 170–230 g, 230–300 g, 500–800 g, >800 g. This species is less frequently sold fresh, e.g. whole fish, whole gutted fish, fillets with skin and skinned fillets (FAO, 2009). Pike-perch successfully cultivated in the RAS by several European companies (Philipsen, 2008; The David Suzuki Foundation, 2008). However, according to accumulated experience, breeding technology is still poor and only few countries (such as Denmark and the Netherlands) have established production. In Russia, pike-perch farming was conducted during the Soviet times. It was a traditional pond aquaculture, and the fish reared on such fish farm cannot be considered as eco-friendly product nowadays. Recently, in the Kaliningrad region we have made some attempts on pike-perch cultivation in the RAS. During the period from 2007 to 2013 we are managed to form brood stock and develop methods of work with a pike-perch from juvenile to commodity output in a closed system. This is the first Russian experience of pike-perch cultivation in the RAS. The growth of pike-perch in natural reservoirs has been studied appreciably well, but the growth in artificial conditions was not detailed or comprehensive enough. Our goal was to study the growth features of pike-perch at different stages of cultivation process. We researched the growth features of various pike-perch generations reared experimentally in a local fish hatchery. We provided an overview of the current status of pike-perch farming in the Russian Federation with a focus on quality of fish, which is produced in environmentally friendly way by using the RAS technology.
Materials and Methods
The objects of the study were a pike-perch of different generations. The research work was carried out on an experimental RAS at the KMP Aqua Ltd. (Svetly, Kaliningrad region) and industrial RAS at the TPK Baltpticeprom Ltd. (Kaliningrad), during the period from 2007 to 2013. Both plants consisted of biofilters (loaded by pelleted polyethylene), mechanical filters and UV lamps. At different stages of cultivation, the fish were distributed in tanks with volume from 700 L to 7000 L. Oxygen levels in the tanks were maintained by Atlas Capco GX11FF air compressor. Scheme of the plant is presented on Figure 1.

Such type of plant can be considered as environmentally friendly. There are no any harmful emissions and we had a full control of outputs and effluents. Oxygen levels in the tanks were maintained above 125% of saturation. The volume of daily replaced water was 10%.

Aller Aqua (Christiansfeld, Denmark) recipes: Aller Futura, Aller Trident, Aller Bronze and Coppens MariCo Focus (Helmond, Netherlands) feeds are used for feeding. All recipes of Aller Aqua contain raw materials ensure an excellent amino acid profile for good metabolism. The composition includes fish meal, fish oil, wheat, vegetable oils, vitamins and mineral supplements. Aller Futura additionally includes krill meal, while Aller Bronze contains soy flour. Coppens MariCo Focus is a high protein low fat grower with a high proportion of fish meal, additionally includes vitamins and minerals.

Daily ration was changing in accordance with the feed consumption. Estimation of feed conversion ratio (FCR) was performed per months by the equalation:

\[
\text{FCR} = \frac{\text{Feed consumption}}{\text{Body weight gain}}
\]  

Temperature maintenance of water in the tank was maintained by heating panels in the plant placement.

To characterize the efficiency of cultivation has been chosen the index of weight gain as the most convenient and commonly used in aquaculture (Купицкий, 2007). Sample size during the control analysis was 1% of the total fish amount in a tank.

Results and Discussion
To date, we have received a third generation of pike-perch which has been farmed under the RAS conditions. At cultivation of brood stock we have achieved the weight of fish in 1–1.3 kg in 34 months period. Weight gain velocity of subsequent pike perch generations was higher than that expected for the first generation due to optimization of biotechnological techniques, especially the feeding regime (Fig. 2).

By the end of the second year of cultivation the pike-perch of third generation reached the weight of 600 g, while the first generation was 460 g. It can be easily seen that the marked differences in the growth rate of pike-perch could be traced from the age of 12–18 months, in particular for its disclosure completeness of growth potency at this age.

During the growing period of third generation, the economical consumption of nutrients to weight gain has been noted. Comparing these data with pike-perch growth from the Curonian Lagoon, it should be emphasized that the growth rate of cultivated pike perch was 2.5 times higher than that of natural pike perch inhabiting the Curonian Lagoon, suggesting the feasibility of cultivation.

The reason why the growth rate of the third generation were higher is optimization of biotechnological techniques, especially the feeding regime as well as a
continual and careful updating of feeding norms in accordance with specific conditions (temperature, oxygen concentration, and configuration and operational features of the RAS, etc.). The feed conversion ratio was below or close to 1.0, thus made a quite effective feeding of the third generation in the described period. This is also can be attributed to the use of high quality feed (Coppens MariCo Focus) at the last stages of cultivation. A high ratio of protein, energy and low fat contend in the feed is reflected in a higher growth rate of fish, which correspond to lower values of feed conversion ratio. The feed containing 10–12% fat is perfect (Schulz et al., 2007) for the pike-perch. The survival rate of various pike-perch generations from larvae to market size fish was about 56%, which is comparable and sometimes exceeds the results obtained by European fish farmers (Хрусталев, Дельмухаметов, 2010). Fishes were produced without hormones, antibiotics, etc., which can be considered as eco-friendly product, especially noting that we had a full control of outputs. Usage of extruded feeds (Aller Trident, Aller Bronze) had no harmful effect on the body composition of fish. The cost price for product was 3.5 euros per kg. Despite this fact, the first batch of product (3,000 kg) was sold in January–February of 2014 very quickly, which indicates a high consumer demand. It should be noted that the consumer demand for pike-perch can increased and depends on fishing ban periods and freezing period. Thus, in the third generation of cultivated pike-perch we have achieved better disclosure of growth potency as compared to the first generation, to ensure higher results in the future.

Conclusions

The main feature of this research in the fact that the cultivation of pike-perch in a closed aquaculture system held in the Russian Federation for the first time. As the results showed, the feasibility of cultivation is very high, that allowing the use our biotechnology in the future. Despite this fact, there is not a fully perfected technique with larvae and fry, especially in survival rate. Terms of pike perch cultivation to market size should be reduced and selected more optimal feed regime for the rearing of marketable fish, and the production must be made cheaper. All these issues will be perspective areas of consideration for the further research.

Acknowledgment

We wish to thank an aquaculture department of Kaliningrad State Technical University for their great help during the research work.

References

3. Available at: http://www.transparencymarketresearch.com/aquaculture-market.html
SHORT COMUNICATION

DETERMINATION OF AFLATOXIN CONTAMINATION IN SOME DRIED NUTS AND SPICES BY ELISA

Ismail Yilmaz, Hafize Garipoğlu

Namık Kemal University, Agriculture Faculty, Department of Food Engineering, Campus Street, No:1 Tekirdağ, Turkey, e-mail: iyilmaz@nku.edu.tr

Abstract

This study aimed to determine the total aflatoxin levels in some dried nuts and spices by ELISA (Enzyme-linked Immunosorbant Assay). In this study, 1158 some dried and spices samples (513 hazelnut, 179 pistachio, 244 almond, 52 peanut, 143 walnut, 2 mahlep, 9 sahlep, 8 cinnamon and 8 black pepper) were randomly obtained from markets and spices shops in Istanbul. Total aflatoxin contamination was determined in 156 (30%) of 513 hazelnut, in 93 (51.96%) of 179 pistachio, in 107 (43.85%) of 244 almond, in 23 (55.95%) of 52 walnut, in 23 (44.23%) of 52 peanut, in 2 (100%) of 2 mahlep, in 7 (78.78%) of 9 sahlep, in 6 (75%) of 8 cinnamon and in 6 (75%) of 8 black pepper. Therefore, monitoring of aflatoxins is necessary to ensure that they are not present at levels that may pose health risks to the public and controls should be done strictly and more often by authorities.

Keywords: aflatoxin, nuts, ELISA.

Introduction

Aflatoxins are acutely toxic, immunosuppressive, mutagenic, teratogenic and carcinogenic compounds. The main target organ for toxicity and carcinogenicity is the liver (Aycicek et al. 2005). Aflatoxins are secondary metabolites produced by species of Aspergillus, especially Aspergillus flavus and Aspergillus parasiticus. Aflatoxins easily occur on feeds and foods during growth, harvest or storage (Colak et al., 2006). A review of monitoring studies on the occurrence of aflatoxins in food products has demonstrated that aflatoxins are still being found frequently in food products at levels that are of significant concern for consumer protection (Chun et al., 2007).

The European Commission and Turkish government have set limits for maximum levels of total aflatoxin allowed in groundnuts, nuts, dried fruit and their products. For foods ready for retail sale, these limits are 4 μg kg⁻¹ (total aflatoxins), and for nuts and dried fruit to be subjected to sorting, or other physical treatment, before human consumption or use as an ingredient in foodstuffs the limits stand at 10 μg kg⁻¹ (total aflatoxins) (Anonymous, 2002a).

ELISA is widely used for analysis at the field of clinical chemistry, veterinary and food control. ELISA analysis is convenient for simultaneous determination of contaminants in a large number of samples with relatively low cost and short time (Chun et al., 2007). Mostly, spices are grown in tropical and subtropical regions and harvested in poor sanitary conditions. These improper conditions are convenient for the biosynthesis of aflatoxins. Therefore, growing conditions, harvesting and processing methods, storage conditions and postharvest treatments should be carefully controlled in order to prevent aflatoxin risks due to contaminated spices. In addition, training programs should be presented for producers.

Turkey is the world’s second largest nut producer, with about 25% of the total world output. The major nuts that are produced in Turkey are hazelnuts, pistachio nuts, almonds, peanuts and walnuts. Turkey is the world’s leading hazelnut producer and exporter followed by Italy and the USA and dominates 80% of world hazelnut trade and export process (Exporters Union, 2006). Most of the supply of approximately 210 000 tons per annum is exported to European Union (EU) countries and elsewhere (Exporters Union, 2006). Turkey is also the third main producer of pistachio nuts with 20% of the world production and takes its position among the first 10 countries in almond production.

Nuts may be contaminated and invaded by fungi during their development in the field, during transport or storage, and fungal contamination of nuts may result in the production of mycotoxins (Arrus et al., 2005). Higher contamination risk is expected in those that are being transported for long distances and stored for a long time under unhygienic, unventilated, hot and humid conditions (Basaran, Ozcan, 2009).

The study on the total aflatoxin in some dried nuts and spices is important to ensure the safety of nuts and spices for human consumption. The aim of this study was to determine total aflatoxin concentrations in some dried nuts and spices that some of them were exported to Europe and other countries.

Materials and Methods

A total of 1158 samples of dried nuts and spices (513 hazelnut, 179 pistachio, 244 almond, 52 peanut, 143 walnut, 2 mahlep, 9 sahlep, 8 cinnamon and 8 black pepper) purchased in Istanbul were randomly obtained from markets and spices shops. Samples were stored at 4 °C in plastic bags until the analysis. All samples were analyzed in duplicate and total aflatoxin concentrations were determined by ELISA. According to Ridascreen Aflatoxin Total (Art No.: 4701) test kit manual, 50 μL of the standard solutions or prepared sample in duplicate were added to the wells of microtiter plate. Then 50 μL of the diluted enzyme conjugate and 50 μL of the diluted antibody solution were added to each well. The solution was mixed gently and incubated for 30 min at room temperature (20–25 °C)
in the dark. The unbound conjugate was removed during washing for three times (ELISA Washer ELX 50, Bio-tek Inst.). Afterwards, 100 μL of substrate/chromogen solution was added to each well, mixed gently and incubated for 30 min at room temperature (20–25 °C) in the dark. Then, 100 μL of the stop solution (1 M H2SO4) was added to each well and the absorbance was measured at 450 nm in ELISA plate reader (ELX 800, Bio-tek Inst.). The mean lower detection limit is 0.25 μg kg⁻¹ (Çolak et al., 2006; Anonymous 2002b).

**Results and Discussion**

During the past decades a huge number of scientific papers have demonstrated that the list of raw materials and processed foods actually contaminated by aflatoxins is continuously increasing spanning from peanuts, known to be contaminated by aflatoxins since 60 s, to cereals, coffee, cocoa, dried fruits and spices (Zinedine et al., 2006). Turkey is a leader in the hazelnut production. And hazelnut and its products are among major exported goods of Turkey. Pistachio is important products that have a high commercial value following hazelnuts (Aluç et al., 2005).

In this study, total aflatoxin contamination was determined in 156 (30%) of 513 hazelnut, in 93 (30%) of 179 pistachio, in 107 (30%) of 244 almond, in 52 (30%) of 179 mahlep, in 7 (30%) of 29 sahlep, in 6 (30%) of 19 black pepper, and in 6 (30%) of 19 cinnamon. Total aflatoxin contamination levels of 156 (30%) of 513 hazelnut were found higher than the Turkish and EU legal limit (10 μg kg⁻¹) (Anonymous, 2002a) (Table 2).

On the other hand, Hazır and Çoksöyler (1998) reported that 46 out of 141 red pepper samples (32.6%) contained aflatoxin. In a similar study in Van, Ağaoğlu (1999) found the highest aflatoxin contamination level of 44.0 μg kg⁻¹ in red-scaled pepper.

### Table 1

<table>
<thead>
<tr>
<th>Total aflatoxin (B1, B2, G1, and G2) contents of some dried nuts</th>
<th>Domestic</th>
<th>Exported</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Aflatoxin</strong></td>
<td>Hazelnut</td>
<td>Pistachio</td>
</tr>
<tr>
<td>Not detected</td>
<td>357</td>
<td>86</td>
</tr>
<tr>
<td>&gt;0–0.5 ppb</td>
<td>70.00%</td>
<td>48.04%</td>
</tr>
<tr>
<td>&gt;0.5–1 ppb</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>&gt;1–2 ppb</td>
<td>126</td>
<td>48</td>
</tr>
<tr>
<td>&gt;2–3 ppb</td>
<td>24</td>
<td>33</td>
</tr>
<tr>
<td>&gt;3 ppb</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

In another study performed by Erdogan (2004), it was reported that total aflatoxins was found in 8 red-scaled pepper samples (18.2%) and in 3 red pepper samples (10.7%). Our results were found to be higher than these results. On the other hand, Hazır and Çoksöyler (1998) reported that 46 out of 141 red pepper samples (32.6%) contained aflatoxin. In a similar study in Van, Ağaoğlu (1999) found the highest aflatoxin contamination level of 44.0 μg kg⁻¹ in red-scaled pepper.

Aluç, Aluç (2005) checked 367 samples of pistachio produced in 3 year (2002–2004) and it has emerged that 33 samples exceeded the maximum tolerated level in Turkish and European countries (4 ppb). Total aflatoxin was not detected in 75% of pistachio samples. These results are correlated well with our findings. The results achieved in our study are in agreement with our findings. Abdulkadar et al. (2004) examined total aflatoxin levels in nut from Qatar and found in 23.4% of nut samples with range of 0.53–289 μg kg⁻¹. Aflatoxin contamination was detected in pistachios and peanuts, while other nuts such as almond, cashew nut, walnut and hazel nut were found free from aflatoxins. In walnut samples, the incidence of AFB1 and AFT was 30%. The contamination levels in walnut samples ranged from 0.56 to 2500 lg kg⁻¹ for AFB1 and from 1.24 to 4320 lg kg⁻¹ for AFT, respectively. The average contamination levels of walnut with AFB1 and AFT were 360 and 730 lg kg⁻¹, respectively (Juan et al., 2007). Out of samples, 20% exceeded the maximum limit (2 lg kg⁻¹) fixed for AFB1 by European legislations (European Commission, 2006). Our results are in agreement with the above findings.

### Table 2

<table>
<thead>
<tr>
<th>Total aflatoxin (B1, B2, G1, and G2) contents of some dried nuts and spices</th>
<th>Walnut</th>
<th>Sahlep</th>
<th>Cinnamon</th>
<th>Black pepper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not detected</td>
<td>63</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>&gt;0–0.5 ppb</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>&gt;0.5–1 ppb</td>
<td>27</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>&gt;1–2 ppb</td>
<td>21</td>
<td>4</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>&gt;2–3 ppb</td>
<td>3</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>&gt;3 ppb</td>
<td>28</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Detected samples</td>
<td>143</td>
<td>9</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

In conclusion, aflatoxins continue to pose a health concern via human exposure to contaminated spices and nuts. Aflatoxins cause economic and trade problems at almost every stage of marketing of nuts and spices especially during export. Therefore, monitoring of aflatoxins is necessary to ensure that they are not present at levels that may pose health risks to the public and controls should be done strictly and more often by authorities.
References

5. Anonymous (2002a) Turkish Food Codex. Legislation about determination of maximum levels of certain contaminants in foods. Basbakanlık Basimevi, Ankara
SHORT COMMUNICATION

DETERMINATION OF AFLATOXIN LEVELS IN CASHEWS ON TURKISH MARKETS

Ismail Yılmaz¹, Mehmet Aluc²
¹ Namık Kemal University, Agriculture Faculty, Department of Food Engineering, Campus Street, No. 1, Tekirdağ, Turkey, e-mail: iyilmaz@nku.edu.tr
² Karin foods, Çevrik Mahallesi, Fatih Cadessi No. 5, Cumayeri/ Diözce, Turkey

Abstract

The problem of food contamination with aflatoxin is one of the current concerns and has received a great deal of attention during the last three decades. Aflatoxins are a group of highly toxic secondary metabolic products as some Aspergillus species. Aflatoxins are carcinogenic, mutagenic, teratogenic and immunosuppressive to most animal species and humans. They are considered to be one of the most important food contaminants affecting food safety and public health.

The aim of the study is to determine the level of aflatoxins in cashews on Turkish markets. Samples were analyzed by reverse phase HPLC containing post column derivatization and fluorescence detection after immunoaffinity column clean-up. AOAC (999.07) was used in as the methodology of the study.

In this study, aflatoxin B1 and total aflatoxins (B1+B2+G1+G2) were analyzed in totally 50 samples of cashews. A total of 50 samples of cashew samples were obtained randomly from supermarkets in Istanbul. Aflatoxins were found in 14 out of 50 samples (28%) of cashews (B1: 0.26–0.32 ppb; total: 0.5–0.84 ppb). All positive samples did not exceed the maximum limit of 2 µg kg⁻¹ set by EU regulations for aflatoxin B1 and total aflatoxin (4 µg kg⁻¹). Although the aflatoxin contamination in cashew samples is very low, aflatoxin analysis should be done strictly for human health.

Keywords: aflatoxin, cashew, HPLC.

Introduction

The cashew tree Anacardium occidentale (French.: acajou, anacarde; Spanish.: marañón, caju, acajú) originates from South and Central America (from Brazil to Mexico). The cashew apple is a pseudocarp, and, in botanical terms, is the thickened stem of a fruit which the actual fruit, the cashew nut, is attached. In its originating countries, the pear-shaped cashew apple is eaten fresh. The fruit is distributed throughout the tropics in this form. It was the development of a roasting method to extract the oil from the shell which turned the nut itself into the main product. Cashew nuts are dried before being sold. After the harvest, cashew nuts need to be immediately placed out in the sun to dry, whilst being continually raked over, until the nuts rattle around in their shells (3% rest moisture). In this form, cashew nuts can be stored for up to 2 years, in ideal storage conditions (dry, dark, cool, well-ventilated). Yet they are usually processed within the same year of harvesting (Anonymous, 2000).

Generally, tropical conditions such as high temperatures and moisture, monsoons, unseasonal rains during harvest, and flash floods lead to fungal proliferation and production of aflatoxins. Poor harvesting practices, improper storage, and less than optimal conditions during transport and marketing can also contribute to fungal growth and increase the risk of aflatoxin contamination (Bhat, Vasanthi, 2003).

Mycotoxins are secondary fungal metabolites that contaminate agricultural commodities and can cause diseases or death in humans and animals have a significant economic impact worldwide. Aspergillus is one of the most important large genus genera. More than twenty five genes are involved in pathway synthesized aflatoxins (Pearson et al., 1999).

The most toxic of this group, is the most potent carcinogen known. Because of their high toxicity, the presence of aflatoxins in food commodities is believed to pose a risk to human health (Leszczynska et al., 2000). Aflatoxin B1 (AFB1) is considered as the most dangerous toxic metabolite because of its hepatotoxic, teratogenic, immunosuppressive and mutagenic nature.

Aflatoxins are regulated in more than 75 countries. Currently, the worldwide range of limits for AFB1 and total aflatoxins are 1–20 ng g⁻¹ and 0–35 ng g⁻¹, respectively (FAO, 2004). European Commission regulations set limits for AFB1 and total aflatoxins of 2 and 4 µg kg⁻¹, respectively in groundnuts, nuts, dried fruit and cereals since 1998 (Commission Regulation, 2006).

Milhome et al. (2014) reported that 24 samples (34.3%), among 70 samples analyzed, were detected presence of total aflatoxins. However, only two samples (2.8%) showed contamination levels above limit set by EU regulations (4.0 µg kg⁻¹), during 2010–2012 period. Aflatoxin contaminations ranging from 2 to 4 mg kg⁻¹ were detected in 22 samples analyzed in 2010–2012, however these samples are in accordance with national and international standards. The incidence of aflatoxins above the allowed limit (EU) has decreased in the last years. In the last year of monitoring (2012) no sample was detected high levels (>4.0 µg kg⁻¹) of total aflatoxins (Milhome et al., 2014).

However, few studies cite the incidence of aflatoxin contamination in cashew nuts. The main objective of this study was to investigate the occurrence and distribution of aflatoxin contamination in cashew nuts imported from India and other tropical countries to assess whether levels of these aflatoxins are affecting food safety and public health.
Materials and Methods
A total of 50 samples of cashews commercialized in Istanbul were randomly obtained from markets and bazaars. Samples were stored at 4°C in plastic bags until the analysis. All samples were analyzed in duplicate.

Aflatoxins were analyzed in cashew nuts according to the method described by AOAC (2007) first aflatoxin standard solution (containing 1000 ng B1, 200 ng B2, 1000 ng G1 and 200 ng G2 per mL) were prepared in toluene-acetonitrile (98+2). Working standard solutions were prepared daily from these standard solutions according to Stroka et al. (2000) For the extraction procedure, 50 g of sample was added with 5.0 g of NaCl, extracted 300 mL methanol: water (80:20 v/v) in a blender at high speed for 3 min and filtered through a Whatman filter paper No.4. 10 mL were diluted with 60 mL of phosphate buffered saline (PBS), and 66 mL of the diluted filtrate was applied to the immunoaffinity column (Aflaprep, R-Biopharm) previously conditioned with 10 mL of PBS (flow rate of about 3 mL min⁻¹). The column was washed with 15 mL of water and air was drawn through the column until dry. Aflatoxins were eluted by applying 1.25 mL of methanol to the column. The eluate was diluted with 1.75 mL of water. A 100 μL aliquot was injected onto the HPLC system (Hewlett Packhard 110 HPLC Chromatograph, equipped with a Hewlett Packard 1100 fluorescence detector). Excitation and emission wavelengths were set at 333 and 460 nm, respectively. The eluate passed through a C18 column (5 μm particle size, 250 mm×4.6 mm). The mobile phase was acetonitrile: water : methanol : toluene (17:54:29 v/v/v), and the flow rate was set at 1 mL min⁻¹. The limit of detection of the method was 0.02 μg kg⁻¹. Quantification of each toxin was performed by measuring peak areas at their retention times, and by comparing them with their relevant standard calibration curve. The identity of each toxin was confirmed in all the analyzed samples by injecting sequentially sample extracts and comparing the peak area ratio with their corresponding standard (AOAC, 2007).

Results and Discussion
Due to the contamination of aflatoxins, the nut is considered as a high risk commodity. The problem of aflatoxin contamination is worldwide but in India, the poor harvesting practices, high temperature, high moisture levels and post harvest practices are conducive for fungal growth, proliferation and aflatoxin contamination (Reddy et al. 2011). In Turkey, cashews are imported from India and other tropical countries.

The occurrence of aflatoxins in different types of nuts and nutty products has been reported by several authors from different countries. Chun et al. (2007) found that nut samples were contaminated with aflatoxins (10.6% of incidence) in the range of 0.20–28.2 μg kg⁻¹ in South Korea. In China, peanut was found contaminated with aflatoxins, being on the average level of 80.3 μg kg⁻¹ and the highest level being 437 μg kg⁻¹ (Wang, Liu, 2006). In Turkey, Yentür et al. (2006) reported that AFB1 and total aflatoxins in peanut butter were in the range of 2.06–63.7 ng g⁻¹ and 8.16–75.7 ng g⁻¹, respectively. According to Cheraghali et al. (2007), 11.8% and 7.5% of 10,068 Iran pistachio nut samples were above the maximum tolerated level of AFB1 and total aflatoxins, respectively. Abdulkader et al. (2004) examined total aflatoxin levels in nut from Qatar and found in 23.4% of nut samples with range of 0.53–289 μg kg⁻¹. Aflatoxin contamination was detected in pistachios and peanuts, while other nuts such as almond, cashew nut, walnut and hazel nut were found free from aflatoxins. The incidence and levels of aflatoxins found in this study were relatively low compared to those quoted in the literature.

Table 1

| Aflatoxin (B1) and total aflatoxin (B1, B2, G1, and G2) contents of cashew samples |
|---------------------------------------------|------------------|---------------|
| AFB1 | Total Aflatoxin |
| Not detected | 36 (72%) | 36 (72%) |
| >0-0.5 ppb | 14 (28%) | – |
| >0.5–1 ppb | – | 14 (28%) |
| >1–2 ppb | – | – |
| Detected samples | 14 (28%) | 14 (28%) |
| Total | 50 | 50 |

The present study on assessment of aflatoxin contamination in total cashew nuts has been satisfactorily performed by HPLC. In this study, aflatoxin B1 and total aflatoxin (B1+B2+G1+G2) were analyzed in totally 50 samples of cashew. A total of 50 samples of cashew samples were obtained randomly from supermarkets in Istanbul. Aflatoxin was found in 14 out of 50 samples (28%) of cashew (B1: 0.26–0.32 ppb; total: 0.5–0.84 ppb) (Table 1). Any of the positive samples did not exceed the maximum limit of 2 μg kg⁻¹ set by EU regulations for AFB1 and AFT (4 μg kg⁻¹). Although the aflatoxin contamination in cashew samples is very low, aflatoxin analysis should be done strictly for human health.

References
**SHORT COMMUNICATION**

**NATURAL ANTIOXIDANTS IN BLACK CHOKEBERRY MARC EXTRACTS DEPENDING ON THE EXTRACTION METHOD**

Ance Dandena, Liga Zuka, Maria Kostova

1. Faculty of Food Technology, Latvia University of Agriculture, 2 Liela Street, Jelgava, Latvia, e-mail: dandena@inbox.lv
2. Silvanols Ltd., 2 a Kurbada Street, Riga, Latvia
3. Medical University – Sofia, Bulevard Akademik Ivan Evstratiev Geshov 15, Sofia, Bulgaria

**Abstract**

The aim of this study was to find out the optimal conditions for extraction of natural antioxidants of grinded black chokeberry marc. The marc was extracted using microwave and ultrasound-assisted extraction methods in water/ethanol solutions with different degrees of concentration, using different particle sizes of the marc and different extraction times. Total content of polyphenols and anthocyanins was determined by spectrophotometry. The highest amount of total polyphenols was found in the extracts obtained using the ultrasound method, a 40% ethanol/water solution and a 1 mm diameter particle size extracted for 30 minutes. The microwave method presented the highest amount of anthocyanins in black chokeberry marc extract, using a 70% ethanol/water solution, a 2 mm diameter particles and 20 minute extraction time. It is necessary to find the most effective extraction method in order to develop a production technology for black chokeberry marc extracts rich in natural antioxidants.

**Keywords:** black chokeberry, natural antioxidants, polyphenols, anthocyanins.

**Introduction**

Black chokeberry (*Aronia melanocarpa*, Elliott) is a natural, rich source of phenolic antioxidants, such as anthocyanins, quercetin derivatives, and, in smaller amounts, vitamin C. Many reports have suggested its anti-proliferative effects against cancer cells, as well as antimutagenic, hepatoprotective, cardioprotective, and anti-diabetic activities. Moreover, a series of papers reported the antioxidant properties of black chokeberry extracts or their phenolic constituents, using various, well established in vitro and in vivo models for direct antioxidant capacity, as well as their protective effects against oxidative stress. Recently, the neuroprotective effects of cyanidin-3-O-glycosides, commonly present in black chokeberry, have been tested in mice. (Gironés-Vilaplana, 2012)

The solid waste streams generated during fruit juice production can be alternative and attractive sources of valuable bioactive compounds due to their low cost and biorenewable nature. The utilization of wastes represents a sustainable approach to the integral benefit of raw materials minimizing the environmental problems caused by their disposal.

Berries contain high levels of phytochemicals with phenolic structure (phenolic acids, flavonoids, hydrolysable and condensed tannins) that can act as antioxidants and have health-promoting activities. The solid pressing wastes originated during separation of peels, seeds and pulp from the fruit juice are an abundant source of flavonoids, colour pigments and pectins. Berries are rich sources of anthocyanins, which impact the dark red or blue colour to the fruit, and are strong antioxidants (Laroze at al., 2010).

The phenolic content and composition greatly differs with the type of berry, and the extraction yield is greatly affected by the solvent. The recovery of compounds from the solid residue after berry processing into juice has been investigated using both conventional and alternative technologies, such as ultrasound or microwave assisted alcoholic extraction. It is widely known that the efficiency of solid/liquid extraction processes is affected by critical processing parameters, such as temperature, nature of solvent, structure of solid matrix (mainly particle size) and extraction time. This means that each plant matrix/ extraction solvent pair behaves in a unique way, so it should be studied as such. On the other hand, both the particle size of the plant matrix and the temperature on the extraction process are easily manipulated physical conditions. In general, a smaller size and a higher temperature facilitate mass transfer, but quantification of such heuristic rules for each plant source is required before optimisation efforts can be rationally developed (Giao et al., 2009).

The present study was aimed at evaluating the effect of extraction method and particle size on the extraction and recovery of antioxidant compounds remaining in the pressing pomace of black chokeberry.

**Materials and Methods**

Fruit pomace of black chokeberry, grown in Latvia, was dehydrated for three days at +50 °C and milled. The ground material was passed through a sieve with different meshes. The fractions were separated and packed in polyethylene bags and kept in dark place at room temperature before use. Solid-solvent ratio 1 : 5. All the samples were assayed in triplicate.

The pressing pomaces from berries were ground in a coffee grinder, and four different particle sizes (0.5, 1, 2 and 3 mm) were selected to evaluate the effect on the yield extraction. Different ethanol solutions were used for the pomace extraction using microwave (70% and 40%) and ultrasound-assisted (50% and 40%) extraction methods. Extraction lasted for 30 minutes at 30 °C with ultrasound bath Elmasonic S30H, 50/60 Hz and 1–20 minutes for microwave oven at 90 W (for longer exposure time samples were already boiling).
Total phenolic content of the extracts were measured using Folin-Ciocalteu method as described by Almey et al. (2010). Gallic acid was used as standard. 0.5 mg·mL⁻¹ stock standard solution of gallic acid was prepared by dissolving 35 mg of dry gallic acid in 50 mL of 70% ethanol and then diluted to 100 mL with the same solvent. Working standards of between 0.0014 and 0.007 mg·mL⁻¹ were prepared by diluting the stock solution with distilled water, adding 2.5 mL of Folin-Ciocalteu reagent (previously diluted 10-fold with deionised water) and after 2 minutes adding 2 mL of 7.5% sodium carbonate solution. Samples were prepared likewise from the extract stock solution prepared at concentration of 2–4 mg·mL⁻¹ (2 mL of sample solution was taken for 25 mL test tube).

All the samples were incubated at 50 °C in water bath for 15 min, cooled quickly at cold water and filled up with purified water. Then the absorbance was read at 760 nm using Camspec M550 spectrophotometer. The standard calibration curve of gallic acid (0.0014–0.007 mg·mL⁻¹) was plotted.

The pH differential method was conducted for detection of total anthocyanin content as described in detail by Lee et al. (2005) using a spectrophotometer. It is based on the structural change of the anthocyanin chromophore between pH 1.0 and 4.5. The difference in the absorbance of the pigments at 520 nm is proportional to the pigment concentration. Results are expressed on a cyanidin-3-glucoside basis. Degraded anthocyanins in the polymeric form are resistant to colour change regardless of pH and are not included in the measurements because they absorb at pH 4.5 as well as pH 1.0.

Results and Discussion

Total polyphenols extraction by ultrasound-assisted extraction using different particle sizes lasted for 30 minutes at 30 °C temperature and showed the highest concentration (60±1 GAE g⁻¹) when 40% ethanol solution and 1 mm particle size was used (Figure 1).

However almost the same concentration (59±2 GAE g⁻¹) was reached with 50% ethanol solution and 1 mm particle size. Meanwhile anthocyanins were extracted and the highest amount of total monomeric anthocyanins (4594 ± 412 mg·L⁻¹) was found at 50% ethanol extract when 1 mm particles were used (Figure 2). The extraction solvent concentration was more important in this case comparing to polyphenol extraction. The most effective extraction in both experiments was obtained using 1 mm particle size of black chokeberry marc.

![Figure 1. Relevance of particle size and polyphenol content by ultrasound-assisted extraction (30 min)](image1.png)

![Figure 2. Relevance of particle size and anthocyanin content by ultrasound-assisted extraction (30 min)](image2.png)

The results obtained using microwaves showed at figures 3 and 4. Two particle sizes (2 mm and 3 mm) and two ethanol concentrations (40% and 70%) were investigated.

![Figure 3. Polyphenol content depending on the extraction time by microwave-assisted extraction](image3.png)
Microwave technology was more effective for 2 mm than 3 mm particles and 70% ethanol was more effective than 40% ethanol solution for both – polyphenols and anthocyanins. Some authors (Landbo, Mayer, 2001; Wang et al., 2011) investigated extraction efficiency using different solvents, temperature conditions, solvent-solid ratios and particle size and concluded that higher yields in extraction resulted from a decrease in particle size. Other researcher groups working with plant material found out that the most effective extraction is achieved when 0.2 mm (Giao et al., 2009; Goula 2013) and <0.5 mm (Fonseca et al., 2006) particle sizes were used. However smaller particles than 0.5 mm were not investigated in this research due to the available meshes, 1 mm particles were more effective than 0.5 mm. Researcher group investigating black chokeberry found >2 mm particles to be the most effective for ultrasound extraction of polyphenols (d’Alessandro et al, 2012), but it was reached in water extraction at 60 °C temperature and solid-solvent ratio 1:20. Hence each plant material and extraction solvent pair behaves in different way as mentioned above and effect of particle size, time, temperature, solid-solvent ratio, solvent composition and an impact of extraction method need to be evaluated separately. These results are significant for new product developing from black chokeberry juice pressing residues – rich in natural antioxidants.

Conclusions

Polyphenols and anthocyanins were successfully extracted from black chokeberry marc of different particle size, with different extraction methods, different solvent concentrations and different extraction time. The highest polyphenol yield was obtained from the marc having 1 mm particle size in 40% ethanol extract using ultrasound for 30 minutes. The highest anthocyanin yield was obtained from the marc having 2 mm particle size in 70% ethanol by microwave extraction for 20 minutes. Both extraction methods may be combined to find the most effective extraction method for both – polyphenols and anthocyanins.

References

SHORT COMMUNICATION

INFLUENCE OF PRE DRYING TREATMENT ON PHYSICAL PROPERTIES OF CARROTS

Maria João Barroca¹, Susana A. Sério², Raquel P. F. Guíne²,³, Paula M. R. Correia²,³

¹ Department of Biological and Chemical Engineering - Coimbra Institute of Engineering, Coimbra, Portugal
e-mail: mjbarroca@gmail.com
² Department of Food Industry, ESAV, Polytechnic Institute of Viseu, Viseu, Portugal
³ CI&DETS – Polytechnic Institute of Viseu, Viseu, Portugal

Abstract

In the present work, the effect of pre-treatment with ascorbic acid on colour changes and texture was investigated during convective hot air drying at 60°C. The pre-treatments were done at 0.25% and 1% of ascorbic acid with treatment times of 60 and 90 minutes. Changes in colour and total colour difference were evaluated by the CIELab colour system and texture of dried carrots was accessed in terms of hardness, springiness, and cohesiveness. Regarding the total colour difference, soaking carrots in ascorbic acid at both concentrations and treatment times turned out to be an ineffective method for reducing the browning reactions during drying. With respect to textural attributes non-significant differences were observed in springiness and cohesiveness of untreated and pre-treated dried carrots but the hardness increased at a pre-treatment time of 90 minutes.

Keywords: pre-treatment, ascorbic acid, colour, moisture content, texture.

Introduction

Carrot (Daucus carota L.) is one of the most commonly used vegetables for human nutrition due to its pleasant flavour, nutritive value, and great health benefits related to its antioxidant, anticancer, healing, and sedative properties. Carrots are constituted by high vitamin and mineral content and other valuable nutrients presented at lower concentrations (Shivhare et al., 2009).

Although carrots are widely consumed as fresh vegetables, due to their perishable nature, they are also subjected to different processes such as freezing, canning or dehydration to extend their shelf-life for distribution and storage. Drying is one of the oldest methods for the preservation of foods. Deterioration of the chemical and physical properties of dehydrated carrots causes changes in their quality that depend not only on drying conditions but also on the other operations carried out before and after drying (Negi, Roy, 2011). Prior to these processes, some chemical or physical treatments were done to the raw material aimed to inactivate enzymes such as polyphenol oxidases (PPO) and to inhibit some undesirable chemical reactions that cause many adverse changes on the quality of the dried product.

The oxidation of phenolic substrates (natural substances that contribute to the sensorial properties) by polyphenol oxidases (PPO) is one of the major causes of the brown coloration of many fruits and vegetables during ripening, handling, storage, and processing (Queiroz et al., 2008). Other non enzymatic reactions, such as Maillard reaction and caramelization reaction also produce browning pigments (Hiranvarachat et al., 2011).

Ascorbic acid is frequently used for browning control of food products and it has been more effective than its isomer isoascorbic acid. This vitamin acts as an antioxidant because it reduces the o-quinones produced by the catalysis of phenolic compounds by PPO, thus limiting secondary reactions that leads to browning and also contributes to decreasing pH. Since optimum pH to PPO range is 5 to 7.5, lower values inhibit enzymatic activity (Ozoglu, Bayindirli, 2002; Gerrero-Beltrán et al., 2005).

The objective of this work was to study the effects of chemical pre-drying treatment (ascorbic acid) at different concentration/time combinations on some physical properties, namely colour and texture, of dried carrots.

Materials and Methods

Sampling

The carrots used in this study were purchased in a local market, and were hand peeled and cut into slices with thickness of 1cm and diameter of 3 cm.

Pre-treatments before dehydration process

Prior to drying, carrots were pre-treated by the following pre-treatment methods: soaking in aqueous solution of 0.25% ascorbic acid for 60 and 90 min and 1% of ascorbic acid for the same times, at room temperature. Also untreated samples were dried, serving as control. After each pre-treatment, the excess of water on sample surface was superficially removed with paper towel.

Drying experiments

Drying experiments of untreated and treated carrots were carried out in a convection oven at 60 °C, with an air speed of 0.2 m/s, in order to reduce the average moisture of carrots to about 15 % (w/w). The drying time of the samples was approximately 8 hours.

Evaluation of colour

The colour of all samples was measured using a handheld tristimulus colorimeter (Chroma Meter-CR-400, Konica Minolta). For each sample, twenty measures were made at different positions of the sample and the obtained values were compared with those of the fresh sample.
The data were reported as average values of these measurements. The colour changes were calculated as:

\[ ΔL = \frac{L - L_0}{L_0}, \quad Δa^* = \frac{a^* - a_0^*}{a_0^*} \quad \text{and} \quad Δb^* = \frac{b^* - b_0^*}{b_0^*} \]

where \( L \), \( a^* \), and \( b^* \) represent, respectively, the lightness, redness, and yellowness of the samples (the subscript 0 is referred to reference-fresh sample).

The total colour difference (TCD), was the parameter considered for the overall colour difference evaluation between a sample and the reference.

\[ TCD = \sqrt{ΔL^2 + (Δa^*)^2 + (Δb^*)^2} \]

**Texture analysis**
The texture profile analysis (TPA) for all samples was made by a texturometer (TA.XT.Plus from Stable Micro Systems). The texture profile analysis was carried out by two compression cycles between parallel plates performed using a flat 75 mm diameter plunger, with a 5 s period of time between cycles. TPA was performed in 20 samples for each state and the measured textural properties were hardness, springiness, and cohesiveness.

**Results and Discussion**
The moisture content of fresh carrots was 87.02±0.14% (wet basis), that is in the range 80–90% as reported by Prakasha et al. (2004).

Table 1 shows the mean value of moisture content of the dried carrots subjected to pre-treatments. Except for the pre-treatment at 0.25% of ascorbic for 60 minutes, the dryings of the pre-treated samples were similar to those of the untreated samples, all with a drying time 8 hours. Thus, the pre-treatment with ascorbic acid (at both concentrations and time treatment) seems to have no consequence on the effective water diffusivity of carrots during air-drying process as compared with untreated and treated samples.

<table>
<thead>
<tr>
<th>Pre-treatment</th>
<th>Moisture, g 100 g⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>16.47±0.63</td>
</tr>
<tr>
<td>AA–0.25% for 60 min</td>
<td>21.39±2.76</td>
</tr>
<tr>
<td>AA–0.25% for 90 min</td>
<td>15.56±1.23</td>
</tr>
<tr>
<td>AA 1% for 60 min</td>
<td>16.57±0.88</td>
</tr>
<tr>
<td>AA–1% for 90 min</td>
<td>16.78±2.33</td>
</tr>
</tbody>
</table>

AA – ascorbic acid; Values expressed as means of 3 replicates±standard deviation.

The changes of colour of carrots are illustrated in Table 2. It was found that all pre-treatment methods led to positive \( ΔL \) values, which indicates an increase of the brightness of the dried carrots. Except for the treatment at 0.25% of ascorbic acid and treatment time 60 min, the brightness of the pre-treated carrots showed a slight decrease, as compared to the untreated samples. In addition, for both concentrations of ascorbic acid, the increase of treatment time allowed a decrease of approximately 5% in the brightness of pre-dried carrots.

<table>
<thead>
<tr>
<th>Pre-treatment</th>
<th>( ΔL )</th>
<th>( Δa^* )</th>
<th>( Δb^* )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>0.12</td>
<td>0.179</td>
<td>-0.066</td>
</tr>
<tr>
<td>AA–0.25% for 60 min</td>
<td>0.18</td>
<td>-0.001</td>
<td>-0.275</td>
</tr>
<tr>
<td>AA–0.25% for 90 min</td>
<td>0.13</td>
<td>-0.008</td>
<td>-0.249</td>
</tr>
<tr>
<td>AA 1% for 60 min</td>
<td>0.10</td>
<td>0.069</td>
<td>-0.265</td>
</tr>
<tr>
<td>AA–1% for 90 min</td>
<td>0.06</td>
<td>-0.014</td>
<td>-0.252</td>
</tr>
</tbody>
</table>

The drying of untreated carrots led to an increment in \( a^* \) colour coordinate that implied that the dried samples were redder than the fresh ones. The carrots showed a bright red colour, which is desirable. In all dried untreated and treated samples, \( Δb^* \) shows negative values, thus meaning that drying decreases yellowness of the carrots. The ascorbic acid soaking leads to similar decrease of \( Δb^* \) values for both concentration/time combinations.

The total colour change (Figure 1) of untreated dried carrots was 10 and this value increased to around 15 to the samples submitted to the four chemical treatments (values of the chemical-treated samples were statistically similar (p>0.05)). This implies that the chemical pre-treatments did not influence positively the colour of carrots during drying.

In fact, carrots soaked in ascorbic acid became less bright and redder than the untreated samples. Therefore, and in order to reduce the total colour difference, it appears that the most appropriate condition of treatment would be at 1% of ascorbic acid and 90 min of treatment time.

Figure 2 reveals the average values of hardness for carrots untreated and treated with ascorbic acid. The hardness represents the force required to deform the sample in mastication, and it is exerted by compressing the food between the teeth or between the tong and the mouth. The hardness of the untreated dried carrots is 30 N and this value is similar to the ones obtained in the pre-drying treatment for 60 min at 0.25% and 1% of ascorbic acid. Hiranvarachat et al. (2011) observed that the microstructures of untreated hot air carrots.
became rigid due to the collapse of cells and pores of the sample with drying and the soaking carrots in water and citric acid did not affect the microstructure after drying. However, when the treatment time was increased to 90 min the hardness of the treated carrots augmented to nearly 45 N, which induced a less intense change of hardness as compared with the fresh carrots.

From Figure 4 it is possible to conclude that the cohesiveness obtained in untreated and treated carrots was very similar, not being observed statistical differences between the different samples. Thus the pre-treatment with ascorbic had no effect on the cohesiveness of dried carrots.

Conclusions

The present work evaluated the effect of pre-treatments with ascorbic acid prior hot convective drying at 60 °C on colour and textural attributes of dried carrots.

The obtained results enabled to conclude that drying allows the samples to be lighter and less reddish than the fresh carrots.

Regarding the total colour difference, the dried carrots soaked with ascorbic acid at both concentrations and pre-treatment times had a higher value when compared with those of the untreated sample. This means that such pre-treatments had not a positive effect on reducing the browning reactions.

With respect to textural attributes, non-significant differences were observed in springiness and cohesiveness of untreated and pre-treated dried carrots.

The hardness of pre-treated dried carrots with ascorbic acid at 0.25% and 1% during 60 min was similar to that obtained for the untreated sample, but this parameter increased if the pre-treatment time raised to 90 min.

Acknowledgment

The authors thank the Portuguese Foundation for Science and Technology (FCT) and Research Centre C&DETS for financial support (project PEst-OE/CE/ED/UI4016/2014).

References

SHORT COMUNICATION

EGG PASTA (ERİŞTE) PRODUCED FROM WHOLE GRAIN OAT FLOUR

Ömer Güvendi, Erkan Yalçın

Department of Food Engineering, Faculty of Engineering & Architecture, Abant İzzet Baysal University, Gökköy Campus, Bolu, Turkey, e-mail: yalcin_e@iba.edu.tr

Abstract

In this study, egg pastas (eriştes) were produced by incorporating refined or whole grain oat flours (ROF, WOF, respectively) at different levels (25, 50 and 100%; w/w) instead of bread wheat flour (BWF) according to the traditional erişte production. Some chemical and quality properties of eriştes were investigated and compared to the control eriştes prepared with BWF or durum semolina. The highest ash, protein and titration acidity contents were obtained with the erişte prepared with 100% WOF. The brightness (L*) values of the oat incorporated eriştes were lower than the controls. An increasing of WOF level caused gradually an increase in the redness (a*) of eriştes. The yellowness (b*) values of WOF incorporated eriştes were higher than ROF incorporated eriştes and control erişte produced with BWF. Cooking properties of eriştes prepared with oat flours were generally lower than those of controls. Total dietary fiber contents of eriştes prepared with ROF or WOF were in the ranges of 5.29–9.16%, or 8.41–18.25%, respectively. WOF containing uncooked eriştes had higher β-glucan content than those of ROF containing and control ones. β-glucan contents of oat included eriştes were in the range of 0.67–3.35% in uncooked form. Total phenolic compound contents of all cooked eriştes considerably decreased. Antioxidant activities of oat included uncooked eriştes were higher than that of controls.

Keywords: oat; whole grain; egg pasta; erişte; β-glucan.

Introduction

Oats have great potential as a health-promoting raw material in several types of foods. Oats have traditionally been used mainly as oatmeal, bran or flakes, which are used to produce porridge, bread and breakfast cereals. Oats are also used in various forms as an ingredient in pasta, biscuits, snack bars and beverages (Kaukovirta-Norja, Lehtinen, 2008). Oat ingredients can be added into a variety of consumer products to provide health-promoting properties, to adjust the flavor and visual appearance or to achieve technological goals. The health effects associated with oats relate essentially to the total dietary fibre and β-glucan contents of oat products (Kaukovirta-Norja, Lehtinen, 2008). The β-glucan of oat and barley has constantly been shown to lower low-density lipoprotein (LDL) and total cholesterol in concern with elevated cholesterol (Jones, 2008).

Pasta products have been consumed in Mediterranean countries for many centuries and it takes the second place after bread in consumption over the world (Torres et al., 2007). Fresh egg pasta (tagliatelle, fettuccine and tagliolini) is a typical product in Italy. It is made by hydrating durum wheat with water and eggs. After mixing and kneading, sheeting-rolls are used for shaping. Egg pasta, which is also a traditional product in west Black Sea region of Turkey, is usually called as ‘Erişte’. It is generally produced with a homemade style. The main ingredients of erişte are wheat flour, water, salt and egg. Semolina is rarely used in its production. The traditional production method differs in many ways from the industrial production method used in pasta companies. It is well known that production parameters highly affect not only quality characteristics but also sensorial and nutritional properties of pasta products (Petitot et al., 2007; De Zorzi et al., 2007; Manthey, Schorno, 2002; Alamprese et al., 2008). In the present study, erişte was produced by incorporating different levels of whole grain or refined oat flours (WOF, ROF, respectively) instead of bread wheat flour (BWF) in order to develop high fiber and healthy erişte for consumers and to help producers for developing an alternative product. Then, some chemical and quality properties of eriştes were investigated and compared to the control erişte prepared with BWF or durum semolina. Oat incorporated eriştes were also evaluated for some of their nutritional properties such as β-glucan, total dietary fiber, total phenolic compound contents and antioxidant activity.

Materials and Methods

Materials

ROF and WOF were obtained from the cereal flour wholesalers located in the city of Istanbul. Other ingredients including BWF, durum semolina, egg and salt were purchased from local markets. β-glucan and total dietary fiber assay kits were provided from Megazyme International Ireland Limited, Wicklow, Ireland. All other chemicals used in this study were analytical grade.

Methods

In the present study, eriştes were produced by incorporating ROF or WOF at different levels (25, 50 and 100%; w/w) instead of BWF in the formulation. Eriştes were produced according to traditional method that includes mixing of ingredients (2% salt, 70 g egg, 10% durum semolina added on 720 g flour basis) with reasonable amount of water (250–450 mL), sheeting of dough, pre-drying on the sadj (hot metal plate) at around 185 °C for 60–90 seconds, cooling to room temperature, cutting into small strands, final drying at room temperature for 1–3 days and storing in plastic bags. Two controls were produced using BWF and durum semolina. Production of eriştes was carried out.
as replicate. Erişte samples were ground and sieved from 500 µm steel sieve (Retsch, Haan, Germany) for analysis. The remaining parts of erişte samples were kept for testing cooking properties.

Moisture, protein (N×6.25), ash, crude oil and titration acidity of the samples were determined according to the Approved Methods of American Association of Cereal Chemists International (AACCI, 2000) following the Methods of 44-01, 46-12, 08-01, 30-10, 31-01, respectively.

Pigment contents of the samples were determined according to AACCI Standard Method No. 14-50 (AACCI, 2000).

The colour properties of erişte samples were measured using the L*, a*, b* colour space (CIELAB space) with Minolta Spectrophotometer CM-3600d (Tokyo, Japan).

Cooking properties, such as optimum cooking time, volume increase, water absorption, cooking loss, were determined according to AACCI Standard Method No.66-50 (AACCI, 2000). Protein loss was determined in cooking water as spectrophotometrically following the method of Lowry et al. (1951). Total organic matter (TOM), representing the amount of surface material released from cooked erişte into the washing water after rinsing, was determined according to D’Egidio et al. (1982).

Total dietary fiber (TDF, %) and β-glucan (%) contents of eriştes were determined using Megazyme assay kits. Total phenolic compounds (TPC, %) of erişte samples were determined according to Gutfinger (1981). TPC were extracted with dimethyl sulfoxide. For the preparation of cooked eriştes in order to analyze β-glucan and TPC contents, eriştes were cooked at optimum cooking time, after removing excess water, samples were lyophilized, then ground and kept for analysis at 4 °C. Antioxidant activities of eriştes were expressed as DPPH (2,2-diphenyl-1-picyrylhydrazyl) scavenging activity (%) following the method of Yu et al. (2002). The concentration of DPPH in test solution was 25 µM. β-glucan, TDF, TPC and antioxidant activity results were calculated on dry weight basis (dwb).

All analysis was carried out as triplicate. The data was statistically evaluated by using SPSS for Windows (Version 16.0). GLM variance analysis was applied. When significant differences were found, the Tukey’s-b multiple comparison test was used to determine the differences among means.

**Results and Discussion**

The crude oil and protein contents of ROF and WOF were 8.6 and 12.6%, and 7.3 and 14.8% on dwb, respectively. Total dietary fiber and β-glucan contents of ROF and WOF were 8.2 and 1.83%, and 23.2 and 3.77% on dwb, respectively.

The moisture contents of controls and oat incorporated eriştes changed in the range of 12.7–13.6%. The ash contents of oat incorporated eriştes were higher than control ones. The crude oil contents of oat incorporated eriştes were higher than controls and the crude oil contents of both types of eriştes increased with an increasing rate of oat incorporation. The protein contents of controls and oat incorporated eriştes changed in the range of 12.2–14.8%. The protein contents of WOF incorporated eriştes were generally higher than ROF incorporated ones (p<0.05). The titration acidity values of oat included eriştes were higher than control eriştes, and titration acidity value increased with an increasing level of oat flour in both types of eriştes. The highest ash, protein and titration acidity contents were obtained with the erişte sample prepared with 100% WOF (data not presented). The pigment contents of eriştes prepared with both flour types gradually increased with increasing levels of ROF and WOF (p<0.05). The pigment contents of eriştes produced with WOF were higher than the erişte samples produced with ROF and control ones. The highest pigment content was obtained with the sample prepared with 50% WOF among the erişte samples.

The brightness (L*) values of the oat incorporated eriştes were lower than the controls. An increasing of WOF level caused gradually an increase in the redness (a*) of eriştes. The yellowness (b*) values of WOF incorporated eriştes were higher than ROF incorporated eriştes and control erişte produced with BWF. According to the statistical variance analysis results, the flour type affected the L*, a* and b* values, in addition, L* values were significantly affected by oat incorporation level (p<0.05).

In determining pasta quality, cooking properties are important quality constraints. Cooking properties, such as optimum cooking time (min), volume increase (%), water absorption (%), cooking loss (%), protein loss (%) and total organic matter (TOM, %) values of eriştes were studied in this research. It was found that the cooking properties of ROF or WOF including eriştes were generally lower than those of the controls. The optimum cooking times of the controls and oat incorporated eriştes changed in the range of 9.0–12.5 min. The optimum cooking times of oat included eriştes were lower than control ones. Lower optimum cooking time is a desirable for quick preparation of egg pastas. The optimum cooking times of WOF incorporated eriştes decreased while increasing an incorporation level. The highest cooking loss value was obtained with 100% WOF included erişte (data not presented). The protein loss values of WOF incorporated eriştes increased while increasing of WOF incorporation level in the formula (p<0.05).

According to the statistical variance analysis results, the flour type had not any significant effect on cooking loss, protein loss and TOM values (p>0.05). Besides, an incorporation level had significant effect on cooking loss (p<0.05), but it had not any significant effect on protein loss and TOM values (p>0.05). TDF contents of eriştes were determined only in uncooked form. TDF contents of eriştes produced with ROF or WOF were mostly higher than controls (p<0.05). TDF contents of eriştes produced with WOF were higher than that of the erişte produced with
ROF. TDF contents of uncooked erişte prepared with ROF or WOF were in the ranges of 5.29–9.16%, or 8.41–18.25%, respectively. The highest TDF content was determined in 100% WOF incorporated erişte (data not presented). β-glucan contents of erişte were determined in both uncooked and cooked forms. β-glucan contents of oat included erişte were generally higher than control ones in both forms. β-glucan contents of oat included uncooked erişte increased as increasing of oat flour incorporation level. WOF containing erişte had higher β-glucan content than those of ROF containing and control erişte in uncooked form. β-glucan contents of oat flour included erişte were in the range of 0.67–3.35% in uncooked form. The highest β-glucan content was determined in 100% WOF incorporated erişte in its both uncooked and cooked forms. TPC analysis was carried out in both uncooked and cooked eriştes. TPC contents of all cooked eriştes considerably decreased, it could be concluded that cooking affected TPC contents harmfully. Antioxidant activity analysis of erişte samples was carried out in uncooked form only. It was found that antioxidant activities of oat flour included eriştes were higher than that of control ones. In addition, WOF incorporated eriştes had higher antioxidant activity than that of ROF included erişte samples (data not presented).

Conclusions

Erişte is a traditional product and it is usually made of BWF in west Black Sea region of Turkey. In this study, it was shown that erişte could be produced from oat flours by replacing with BWF in different ratios. Besides, it could be produced from 100% ROF or WOF as an alternative dietary fiber rich product. It is expected that oat flour incorporated eriştes will provide important health benefits for consumers and will be substitute product for homemade manufacturers. The results also showed that all oat flour included eriştes can be regarded as good sources of dietary fiber.

Acknowledgment

This work was financially supported by Abant Izzet Baysal University Research Fund (BAP-2010.09.01.337).

References

**SHORT COMUNICATION**

**IMPACT OF LOW TEMPERATURE, PROLONGED TIME TREATMENT AND VACUUM DEPTH ON THE PORCINE MUSCLE QUALITY AND SAFETY**

Alvija Salaseviciene¹, Lina Vaičiulienė², Irina Koscelkovskienė¹

¹ Food Technology Department, Faculty of Technology and Landscaping, University of Applied Sciences, Pramonės pr.22, Kaunas, Lithuania, e-mail: alvija.salaseviciene@go.kauno.lt

² Food Institute, Kaunas University of Technology, Taitos av. 92, Kaunas, Lithuania

**Abstract**

The effect of low temperature (53 °C), long time heat treatment (Tc +5 h and Tc + 17 h – holding time, Tc – core temperature equal to the water bath temperature), and vacuum depth (0.3 and 0.6 ×10⁵ Pa) on texture and microbiological safety parameters in *M. longissimus dorsi* from slaughter pigs were studied. The study involved analysis of meat moisture, toughness, cooking loss and microbiological parameters. Decreasing shear force and increasing cooking loss during low temperature and long time treatment was observed at 53 °C temperature along with the treatment duration (Tc +5 h and Tc +17 h). Positive shear force and vacuum depth correlation was observed in the samples. No correlation between samples moisture content and shear force was found. Microbiological analysis revealed safety of the produced product within two weeks period storage at 4 °C temperature.

**Keywords:** LTLT, vacuum depth, porcine quality and safety.

**Introduction**

Gentle treatment technologies have been improved during the last decade by cook-in-bag / container technology, which has been extensively adopted by catering services and food processing plants due to the possibility of increased consistency along the muscle as well as appealing texture and colour of the meat. Studies have shown that meat is more tender and has less cooking loss when heating temperatures are low. A range of heat-induced changes occur at temperatures between 48 °C and 63 °C, and these events, alone or due to the presence of other proteins can be of significance to toughness, cooking loss and colour of LTLT treated meat (Christensen et al., 2011, Christensen et al., 2013). Clearly temperature, but also duration of cooking has a large effect on the physical properties of meat and the eating quality. But the effect on these physical properties when meat is heated at lower temperatures for a long period of time (LTLT) and different vacuum levels is not clearly understood. The aim of the study was to investigate the combined effect of heating temperature, time and vacuum depth on the physical properties and microbiological stability of LTLT treated *Longissimus dorsi* muscle from slaughter pigs.

**Materials and Methods**

*Samples:* *Longissimus dorsi* (LD) muscles were obtained from both sides of 10 slaughter pigs. Whole muscles were vacuum packed and stored at 4 °C for 2 days. After storage muscles were cut into samples, vacuum packed at different depth of vacuum (0.3×10⁵ Pa and 0.6×10⁵ Pa) and heat treated at 53 °C for Tc +5 h and Tc + 17 h – holding time (Tc – core temperature equal to the water bath temperature). After thermal treatment, samples were taken from the water bath and immersed in an ice-water bath until the temperature reached 26 °C. The samples were then stored in a storage room at 0–4.0 °C for 24 h up to 14 days before analysis. After storage, sample weights were recorded in order to calculate cooking weight losses, samples moisture analysis conducted (105 °C).

*Warner–Bratzler shear force:* blocks of 1×1×6 cm were cut from the heat treated samples. Each block was sheared 3 times by Lloyd TAI Texture Analyzer equipped with a triangular Warner–Bratzler test cell. Mean maximum force required to shear through the samples, Warner–Bratzler Shear Force, was determined from each LTLT treatment.

*Microbiological analysis:* A surface area of 14.7 cm² and 0.3 cm depth was sampled in an aseptic manner from the surface of each muscle (raw, LTLT processed, stored for 7 and 14 days). Homogenates and suitable dilutions were prepared. Total plate count, mesophilic, thermophilic bacteria, *Enterobacteriaceae*, *Escherichia coli* counts, yeast and were performed according to standard procedures: LST EN ISO 4833:2003, LST ISO 21527-1:2008, LST ISO 21528-2:2009, LST ISO 16649-2:2002.

*Statistical analysis* was performed using the Statistical Analysis System (SAS, 1989). Variance was analyzed by using the general linear model procedure of SAS.

**Results and Discussion**

Effect of LTLT treatments in different vacuum depth on cooking weight loss, Warner–Bratzler shear force and water content. This study investigated the combined effect of heating duration of Tc + 5 h and Tc + 17 h and vacuum depth of 0.3 and 0.6×10⁵ Pa of samples treated at 53 °C temperature evaluating in raw and LTLT treated samples moisture, cooking loss, shear force parameters. Table 1 shows the results obtained for cooking weight loss (CL), Warner–Bratzler shear force (WB) and moisture.
Among alterations in the meat occurring during cooking are toughness and juiciness changes, both of them being important when assessing eating quality of meat.

As expected, cooking loss increased when thermal treatment time was prolonged from Tc+5 h to Tc+17 h in both samples groups: by 91% for LTLT samples treated in 0.3×10³ Pa vacuum depth, and 68% for samples treated in 0.6×10³ Pa vacuum depth. However no significant differences were found (p>0.05) within the LTLT samples treated the same time but different vacuum depth. The results are in line with those reported for *Semitendinosus* (ST) muscle from young bulls (Christensen et al., 2013), although controversial result of the same authors was found for *Longissimus dorsi* muscle from slaughter pigs where increasing time did not affect the cooking loss at any temperature (Christensen et al., 2011).

Moisture content did not differ significantly, except for LTLT samples treated for Tc + 17 h at 0.6×10³ Pa vacuum depth, where moisture was 11% lower than the average of LTLT treated samples. Cristensen et al. (2012) found that juiciness decreased with increasing heating temperature and time in all species, and cooking loss increased with increasing temperature.

Shear force (WB) mean values increased as the vacuum depth increased, significant differences (p<0.05) being observed between the mean values obtained in samples treated for 5 h in 0.3 and 0.6×10³ Pa vacuum depth where shear force increased by 69% and by 23 % in samples treated of 0.3 and 0.6×10³ Pa for 17 h. Both heating time and vacuum depth significantly affected shear force for LTLT porcine samples treated for 5 h at 53 °C temperature, the linear relationship was found for the experiment, although for 17 hours treated LTLT samples there was inverse effect registered between vacuum depth and shear force parameters (Table 1). Mortensen et al. (2012) performed sensory analysis of eating quality and found increased tenderness of ST from young bulls with increasing heating times from 3 to 12 h at 56 °C, 58 °C and 60 °C, although in contrast to the results of the current decreased shear force with increasing heating time was found in cows, where WB-PF of ST decreased with increasing temperature and only minor decreases of WB-PF were observed with increased heating time, except at 55 °C where a significant decrease in WB-PF was observed with increasing temperature from 2.5 h to 19.5 h (Christensen et al., 2013).

**Effect of LTLT treatments on product microbiological quality**

The temperature and treatment duration primarily defines the sensory quality, microbial safety and shelf-life of LTLT products. A number of national and international recommendations exist, such as good manufacturing practice (GMP). Significant process parameter differences are evident among the various GMP recommendations; nevertheless, all of them, invariably, focus upon microbiological safety (Vaudagna et al., 2002). The results from the current microbiological investigation of the raw meat (LD) and LTLT treated LD from pigs are presented and the effect of 53 °C treatment for 5 and 17 h regime on samples safety discussed below.

Microbiological analysis of raw *M. longissimus dorsi* revealed the pollution status of the raw material ready to use for LTLT treatment. Total plate count varied within the limits of 2.6×10⁶ to 1.1×10⁷ CFU g⁻¹, mesophilic bacterial count – 2.0×10⁶ to 0.8×10⁷ CFU g⁻¹, thermophilic bacteria count – 1.0×10⁴ to 5.1×10⁵ CFU g⁻¹, yeast and mold count within the limits of 3.8×10² to 6.0×10² CFU g⁻¹, spores of thermophilic microorganisms, *Enterobacteriaceae* and *E. coli* have not been identified in the samples. Microbiological data suggested that the initial contamination of the raw material was high enough, but no defects and deterioration of the samples were observed. Bacterial spores that can survive heat treatment have not been found.

Treatment of 53 °C temperature for 8 h (Tc +5 h) and vacuum depth of 0.3×10³ Pa and 0.6×10³ Pa significantly diminished microbiological contamination in the LTLT treated samples within the 1st shelf life day compared to those of raw meat samples. Samples treated with different vacuum depth of 0.3×10³ Pa and 0.6×10³ Pa revealed that total plate count varied within the limits of 1.6×10² and 2.0×10⁴ CFU g⁻¹, mesophilic bacteria count – 1.1×10⁶ and 1.4×10⁷ CFU g⁻¹, thermophilic bacteria count within the limits of 7.5×10³ and 4.3×10⁵ CFU g⁻¹ respectively. Yeast and molds, mesophilic and thermophilic bacteria spores, *Enterobacteriaceae* and *E. coli* have been identified in the LTLT samples.

LTLT samples (treatment of 53 °C temperature for 8 h (Tc +5 h) and vacuum depth of 0.3×10³ Pa and 0.6×10³ Pa) were kept for 14 days and analysed for microbiological contamination on the 7th and 14th day of the storage. A minor growth of micro-organisms in the samples stored for 7 days was registered; however

---

**Table 1**

| Physical properties analysis of LTLT *Longissimus dorsi* muscle (mean values) |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Treatment / Properties          | Raw meat        | Tc+5h, 0.3 10³Pa | Tc+5h, 0.6 10³Pa | TT,+17h, 0.3 10³Pa | TT,+17h, 0.6 10³Pa |
| Moisture, %                     | 664.24          | 667.76          | 667.88          | 667.99          | 557.76          |
| Share force, N                  | 552.43          | 449.90          | 884.24          | 559.38          | 772.34          |
| Cooking loss, %                 | NA*             | 88.72           | 99.02           | 116.66          | 115.12          |

*NA-not applicable*
significant microorganisms’ increase (with no threat to human safety) in the samples kept for 14 days was identified. Samples treated with different vacuum depth of $0.3 \times 10^3 \text{ Pa}$ and $0.6 \times 10^3 \text{ Pa}$ revealed that total plate count varied within the limits of $3.2 \times 10^4$ and $3.0 \times 10^3 \text{ CFU g}^{-1}$, mesophilic bacterial count – $2.7 \times 10^8$ and $1.4 \times 10^8 \text{ CFU g}^{-1}$; thermophilic bacteria count - $1.1 \times 10^4$ and $0.8 \times 10^5 \text{ CFU/g}$ respectively. Yeast and molds, mesophilic and thermophilic bacteria spores, Enterobacteriaceae and E. coli have not been identified in the LTLT samples within the 14 days of storage.

Treatment of $53^\circ\text{C}$ temperature for $20 \text{ h}$ ($\text{TC} + 17 \text{ h}$) and vacuum depth of $0.3 \times 10^3 \text{ Pa}$ and $0.6 \times 10^3 \text{ Pa}$ significantly diminished (by 10 times) microbiological pollution in LTLT samples compared those of raw meat samples within 7 days of storage. The results revealed that microbiological indicators level was similar to those of $8 \text{ h}$ ($\text{TC} + 5 \text{ h}$) treatment within the whole storage period (14 days).

Both LTLT treatments for $8$ and $20 \text{ h}$ duration with different vacuum depth enabled to reduce the marginal pollution of raw meat samples from $2.6 \times 10^6$–$1.1 \times 10^7 \text{ CFU g}^{-1}$ to $2.0 \times 10^3$–$3.5 \times 10^3 \text{ CFU g}^{-1}$ in LTLT samples already at the 1st day of storage, with inactivation of yeast and molds.

Conclusions

Application of low temperature and long time treatment ($53^\circ\text{C}$, $\text{TC} + 5 \text{ h}$ and $\text{TC} + 17 \text{ h}$ – holding time, $\text{TC}$ – core temperature equal to the water bath temperature, and vacuum depth of 0.3 and 0.6 $\times 10^3 \text{ Pa}$) revealed that:

- cooking loss increased due to low temperature treatment prolongation from $\text{TC} + 5 \text{ h}$ to $\text{TC} + 17 \text{ h}$ in LTLT $\text{M.Longissimus dorsi}$ samples;
- shear force (WB) mean values increased as the vacuum depth increased, significant differences ($p<0.05$) being observed between the mean values obtained in samples treated for $5 \text{ h}$ in $0.3$ and $0.6 \times 10^3 \text{ Pa}$ vacuum depth where shear force increased by 69% and in samples treated of $0.3$ and $0.6 \times 10^3 \text{ Pa}$ for $17 \text{ h}$ by 23%.

Analyzing the obtained microbiological analysis results it can be concluded that employing LTLT treatment and different depth of the vacuum ($0.3 \times 10^3 \text{ Pa}$ and $0.6 \times 10^3 \text{ Pa}$) the microbiological safety of the LTLT product was ensured for a maximum period of 14 days, as longer storage times would increase the risk of mesophilic and thermophilic bacteria development.

References

SHORT COMMUNICATION

STANDARD METHODS AND CRITERIA TO PREDICT BREAD CEREAL QUALITY – DO THEY STILL MEET THE DEMANDS OF MODERN RAW MATERIALS AND THEIR PROCESSING?

Meinolf G. Lindhauer
Department of Safety and Quality of Cereals, Federal Research Institute for Nutrition and Food, Max Rubner-Institute (MRI), Schuetzenberg 12, D-32756 Detmold, Germany, e-mail: meinolf.lindhauer@mri.bund.de

Abstract

Reliable prediction of the baking behaviour of bread cereals (wheat, rye) is of utmost importance in the respective production, trade and processing. Consequently, there is a great interest in easy to handle, rapid and precise methods. A broad variety of such methods has been established by international standardisation organisations like ICC, AACCI, ISO and CEN, respectively, to assess a wide range of criteria of grain raw material and products thereof. In wheat because of its gluten functionality in baking performance protein quantity and quality assessment criteria play a decisive role. In addition, starch characteristics may be of significance.

In this respect, it has become tradition in practical wheat trade and buying decision making to measure raw protein content (by the Kjeldahl or Dumas method, preferably by NIR), protein quality (as sedimentation value), and Falling number, only. Data received are then put into relation to the bread volume tentatively to be expected. In rye starch and pentosan quantity and quality play the crucial role in baking performance. Thus, the amylogram data gelatinisation maximum and gelatinisation temperature serve as criteria of quality prediction. The presentation will show that progress in wheat and rye breeding on the one hand and the increased diversity in baked goods on the market have made the prediction reliability of traditional methods questionable, at least in specific cases. Furthermore, the technical procedure of performing individual methods will have to be evaluated to avoid misleading results.

Keywords: Wheat, rye, baking quality, standard methods.

Introduction

On different levels of the bread cereal (wheat, rye) production and utilization chain it is of utmost interest to sufficiently predict the tentative processing, i.e. baking quality of the raw material. In early stages of their selection process breeders would like to know if the wheat or rye material they work with will finally fulfil the demands of the processing industry after a ten to 14 years breeding and variety releasing process. Cereal traders and/or millers are permanently facing the challenge to select the right quality material they are aiming at for their specific interest and bakers, finally, need to know the specific processing quality of the milling products they ordered. Therefore, since many decades cereal scientists in close cooperation with practice have elaborated and established a system of methods suitable for mostly rapidly predicting the processing, especially baking performance of a raw material lot of interest and/or a milling product prepared thereof. Basically a baking test would mirror best and most comprehensively the real processing (baking) quality. However, baking tests afford specific and expensive equipment and infrastructure and they are rather time consuming and thus not suitable to be practised under time constriction during harvest and/or primary cereal uptake in mills or trading points.

As a consequence a broad range of so-called indirect methods has been established the results of which are put into relation to a specific dough making and subsequent baking performance thus enabling prediction of the baking result. To facilitate international cereal trade a wide range of methods has been standardised by international standardisation organisations such as the International Association of Cereal Science and Technology (ICC), the American Association of Cereal Chemists International (AACC), the International Standardisation Organisation (ISO) and the Comité Européen de Normalisation (CEN).

As in wheat baking performance is to a high extent caused by its protein, specifically by its gluten fraction and by the starch fraction, specific (rapid)methods are designed to measure protein (or gluten) quantity and quality, respectively, and starch quality. In rye, the baking quality of which is characterized predominantly by the carbohydrate fraction, i.e. starch and pentosas. Consequently, methods aim at describing this fraction in its dough and gel forming capacity. For a long period of time it had become practical use in wheat evaluation – at least in the phase of primary uptake of cereal material at harvest – to measure total protein content and sedimentation value as protein (gluten) quality criterion and in addition Hagberg-Perten Falling Number to get information about the starch. This had turned out to be sufficient as the wheat variety material so far on the market showed a good linear relationship between protein characteristics and baking performance mostly taken as volume of the baked good. In specific years with pre-harvest sprouting Falling number was of additional significance.

In rye, more susceptible to pre-harvest sprouting, Falling Number and starch gelatinisation patterns as measured by means of the Brabender amylograph equipment, i.e. starch gelatinisation maximum and temperature at gelatinisation maximum were sufficient to handle rye milling products for bread making. In most recent years, however, there is – at least in Germany – more and more complaining among wheat and rye breeders, farmers, traders and millers that these traditional methods are no longer sufficient to predict...
wheat and rye baking quality. This is specifically conferred to modern German wheat varieties and to a certain extent to rye cultivars it is the intention of the following to describe the given situation in Germany and to consider the question of still existing significance of the established quality assessment system.

**Material and Methods**

Data for bread wheat (*Triticum aestivum*) and rye (*Secale cereale*) are derived from the official yearly German quality assessments of bread cereals performed by law by Max Rubner-Institute (MRI) - Department of Safety and Quality of Cereals during recent years as well as from processing quality assessments performed in the framework of the releasing process for new varieties. Analytical methods applied are according to the ICC standardization protocol as follows (ICC 2001): Total protein (ICC Standard No. 105/2), sedimentation value (ICC Standard No. 116/1), falling number (ICC Standard No. 107/1). As baking test the RMT (Rapid Mix Test) with rolls has been chosen being the respective standard procedure in Germany (Arbeitsgemeinschaft Getreideforschung e.V., 1994). For quality assessment of rye the Brabender Amylograph procedure (ICC Standard No. 126/1) was applied.

**Results and Discussion**

With respect to baking quality much progress in breeding has been made during the last decades with wheat rather than with rye. Therefore, emphasis is laid in the following on wheat. In Germany – and to a certain extent in other countries too – it has been well established in the wheat business to look at raw protein content as measure of protein quantity. And as there is sufficiently narrow relation protein content enabled anticipating the gluten content of a wheat sample. As a certain measure of protein quality sedimentation value added further information. Starch characteristics, i.e. it's gelatinization capacity and indirectly the sample's status of amylase activity could (and of course, can) be derived from falling number measurements, the significance of these data being of utmost relevance in harvest years with pre-harvest sprouting as a problem. Taking this into account the "3 pillars" - protein content, sedimentation value, and falling number – were mostly sufficient to evaluate the baking quality of a wheat sample (Fig. 1). This was especially due if the variety or a blend of varieties in a sample were known.

As for a long time until the late nineties of the last century and the early years of the 21st century there mostly was a positive linear relationship between raw protein content and baking quality in the sense of volume forming in yeast leavened baked goods. Therefore it had become use to measure total protein content of a wheat sample, only, and to evaluate from this the baking quality to be expected. This procedure had been supported by the fact that it more and more easy to measure protein content rapidly by near-infrared spectroscopy (NIR). Consequently, it had become tradition internationally to pay wheat quality for its protein content.

**RMT-volume estimation by the Bolling (1969) regression equation**

**Basis : Grain protein content : Sedimentation value**

\[
\text{Volume yield} = 10 \times \text{protein} + 3 \times \text{sedimentation value} + 420
\]

(valid for E and A wheats)

\[
\text{Volume yield} = 17 \times \text{protein} + 3 \times \text{sedimentation value} + 306
\]

(valid for B wheat)

Fig. 2: Regression equations to estimate Rapid-Mix-Test (RMT) volume of wheat sample

More recently, however, more and more wheat cultivars have been released where obviously the narrow relationship between increasing protein content and increasing baking volume does no longer exist. This is to be demonstrated in Figure 3, showing the cultivars Pamier and Kranich representing the "old" relationships, Toras and Tarso, on the contrary, do only have a very weak relationship between protein content and baking performance. Reliable prediction of the baking quality of a wheat sample of these varieties or containing these varieties has become a challenge.
Fig. 3: Protein content/RMT baking volume interactions in selected German wheat varieties

Baking quality evaluation of wheat has become additionally more complicated as there are modern varieties on the market that show relatively low total protein amounts (and gluten quantities, respectively) but because of the high functionality of the gluten proteins the baking performance of such wheat is much better than expected from classical measurement results. Also it may be necessary to review the sedimentation value method as it can be shown that results alter in dependence on the milling equipment used to prepare the flours resulting into different protein content of the test sample flours (Selting, unpublished). Low falling number values are traditionally believed to result in bad baking performance of wheat flour. Samples with low falling number values, however, have been identified during recent harvest years that showed nearly no restriction in baking performance. Own starch related studies with respective samples make assume that starch kernel integrity coinciding with strong gluten functionality are more important than falling number values acquired under conditions of a surplus of boiling water in the FN vessels (Muenzing and Lindhauer, unpublished).

Rye baking quality, finally, is also sometimes questioned as it is believed that flours from modern (German) rye varieties cause problems in baking performance. Specifically, bakers complain about so-called dry baking, i.e. reduced crumb humidity and limited shelf-life of breads. Additionally, crumb cracks are said to occur more often using modern rye varieties than it happened with older varieties. It must remain open, so far, if the traditional criteria gelatinization and temperature at gelatinization maximum derived from the Brabender amylogram are still sufficient for describing the baking quality of modern varieties. Fact is that modern rye varieties tend to be more pre-harvest sprouting resistant and, thus, they tend to have higher falling numbers.

References
ANALYTIC STUDY OF UNIVERSITY STUDENTS' NUTRITIONAL HABITS AND ATTITUDES AS A PART OF SOCIETY IN TURKEY

Yasemin Oraman, Emine Yilmaz, Gokhan Unakitan, Burcin Basaran

Department of Agricultural Economics, Agricultural Faculty, Namik Kemal University, Tekirdag/Turkey,
e-mail: yoramam@nku.edu.tr

Abstract

The importance of proper nutrition as one of the enhancing nutrition attitudes, knowledge and important aspects of lifestyle were emphasized in the practices of students which has high importance, because recent years and the trend towards healthier diets subsequently will lead to more food-conscious increased.

The aim of this research is to understanding the nutrition knowledge, attitude and food habits of students at higher education programmes. This study has been carried out using subjects of 618 students consisting of 237 female and 381 male students attend from the educational programs present at the Namik Kemal University. A Likert type scale was used to evaluate each of the questions. The collected data was analysed by t-test, one-way ANOVA and Pearson correlation coefficient (P<0.05). In addition, this result supported by principal component analysis (PCA), descriptive statistical analysis which students’ attitudes on nutritional habits. In research there was significant differences in the knowledge level between students of different departments (F=3.06; p<0.001). Principal component analysis was used to identify four main dietary patterns, and analysis of variance employed to examine the characteristics associated with them. Factor analysis reduced the 15 independent variables into four factor groups. This paper provides a unique insight into a wide range of nutritional habits among young’s in Turkey (e.g. vegetables, fast foods, milk products etc.-related) and reflects on the responses obtained from policy makers’ towards food habits in Turkey.

Keywords: students' nutritional habits, proper nutrition, eating attitudes, factor analysis.

Introduction

Food and nutrition have an essential role in children and young people's achievement at all stages of education. There is evidence that young people's food choices can affect their attendance and behaviour as well as their health. There have been considerable changes in human lifestyle all over the world in recent decades. The importance of proper nutrition as one of the enhancing nutrition attitudes, knowledge and important aspects of lifestyle were emphasized in the practices of students has high importance, because recent years and the trend toward healthier diets have subsequently lead to more food-conscious individuals.

The main goal of healthy nutrition plans is to obtain the appropriate and necessary nutrition to remain healthy, to be physically prepared and to lead a healthy life. For this reason to promote the health level of a society, and the attitudes of its people, must be taken into account (Azizi et al., 2011). Given that one of the main goals of universities is to broaden the knowledge of the people in a society, the enhancement of the nutrition attitudes, knowledge and practices of its students is of high importance, as this will subsequently lead to a more food conscious society and more healthy people. Some studies have shown that most students are not familiar with the healthy foods needed for their body in different conditions (Cotugna et al., 2005; O’dea, 2004).

Elhassan et al., (2013) was to assess nutrition knowledge, attitude and practices among Ahfad University students. The other research showed that the majority of students (83.6%) eat three meals during the day regularly and no difference was found between men and women (Ruka et al., 2005). O’dea also exposed that 85% of men and 87% of women, who are overweight, decide to go on a diet to lose weight; also13% of men and 20% of women refuse to eat breakfast. He also reported that students do not have the necessary information and training regarding weight control, nutrition needs and diets (O’dea, Abraham, 2001). According to Gates students with normal weight have a more healthy diet and better points in terms of nutrition knowledge and attitudes compared the others (Gates, De Lucia, 1998).

There is no significant difference between knowledge and attitude between overweight and normal weight persons. In normal weight persons, the body mass index and body fat percentage were related to their attitude to nutrition (Lowry et al., 2000; Mitchell et al., 1999).

The aim of this research was to assess the nutrition knowledge, attitude and food habits of students at higher education programmes.

Materials and Methods

The study is a descriptive cross-sectional, community based study. The study included 618 students consisting of 237 female and 381 male students attend from the educational programs present at the Namik Kemal University are faculties of engineering, arts design and architecture sciences, economics and administrative sciences, theology, medicine, veterinary medicine, agriculture, foreign languages, health sciences, and five vocational schools.

The Questionnaire consisted of five parts and first part is about some personal information about students and their body composition. The second part contains questions that measure students’ eating behaviour and its relation to nutrition knowledge. The third part includes some questions about student’s attitudes toward nutrition habits. The forth part poses some questions about choosing the foods and the last part collects student’s recommendations. In two and third parts the Comprehensive Assessment of Nutrition
Knowledge, Attitudes, and Practices CANKAP (Cunningham, Skinner, et al., 1981) test was used. Likert type scales (Triola, 1992) were used for those responses to the items testing nutritional behaviour. The university students identified their eating behaviour using a five-point scale, ranging from “Never”, “Seldom”, “Sometimes,” “Usually,” and “Always”.

The collected data was analysed means, standard deviation and percents were calculated for the scores from the nutrition knowledge, attitude and food habits sections. Pearson’s correlation coefficient were used to assess the correlation between nutrition knowledge, the attitude and analysis of variations (ANOVA) was used to evaluate nutrition knowledge and the attitude between majors, and an independent t-test was used to compare the nutrition knowledge and attitude between males and females. Statistical results were considered to be significant at p≤0.05. In addition, this result supported by principal component analysis (PCA), descriptive statistical analysis which students’ attitudes on nutritional habits.

Results and Discussion

Table 1 shows the basic demographic characteristics of the students.

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>381</td>
<td>61.7</td>
</tr>
<tr>
<td>Female</td>
<td>237</td>
<td>38.3</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-21</td>
<td>483</td>
<td>78.1</td>
</tr>
<tr>
<td>22-25</td>
<td>123</td>
<td>20.0</td>
</tr>
<tr>
<td>Above 26+</td>
<td>12</td>
<td>1.9</td>
</tr>
<tr>
<td>Current place of residence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>urban area</td>
<td>534</td>
<td>86.4</td>
</tr>
<tr>
<td>rural area</td>
<td>84</td>
<td>13.6</td>
</tr>
<tr>
<td>Permanent residence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dormitory</td>
<td>141</td>
<td>22.5</td>
</tr>
<tr>
<td>at home with their friends</td>
<td>405</td>
<td>65.5</td>
</tr>
<tr>
<td>with family</td>
<td>51</td>
<td>8.3</td>
</tr>
<tr>
<td>with a relative</td>
<td>21</td>
<td>3.4</td>
</tr>
<tr>
<td>Family income</td>
<td></td>
<td></td>
</tr>
<tr>
<td>less than 1000 TL</td>
<td>237</td>
<td>38.4</td>
</tr>
<tr>
<td>1000-2000 TL</td>
<td>273</td>
<td>44.17</td>
</tr>
<tr>
<td>more than 2500TL</td>
<td>108</td>
<td>17.48</td>
</tr>
</tbody>
</table>

Most (61.7%) of the participants were male. The mean age of the students was 20.5 years; nearly 78.1% of the total participants were aged between 18 and 21 years. The family income of the majority of students was more than 2500 TL (17.48%), then between 1000 and 2000 TL (44.17%), then less than 1000 (38.4%). The mean values and standard deviation for age, height, weight and the body mass index (BMI) of the students are shown in Tables 2 and 3.

It is known that age has a central role in the potential to improve especially the dietary habits and lifestyle behaviours of children and young adolescents and improving nutrition knowledge in young people may translate into educating them in good dietary habits. In order to promote healthy nutritional behaviours and prevent overweight and obesity, it is important to target this population with interventions concerning their eating habits and lifestyles (Grosso et al., 2012).

<table>
<thead>
<tr>
<th>Anthropometric characteristics of the students</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
</tr>
<tr>
<td>--------</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Body mass index of the students</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
</tr>
<tr>
<td>Number (%)</td>
</tr>
<tr>
<td>Thinness (less than 20 kg/m²)</td>
</tr>
<tr>
<td>Normal weight (20-25 kg/m²)</td>
</tr>
<tr>
<td>Overweight (25-30 kg/m²)</td>
</tr>
<tr>
<td>Obese (More than 30 kg/m²)</td>
</tr>
<tr>
<td>No reply</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

The results concerning nutrition practices also showed that 27.2% of the participants eat fish once or twice a month. In addition, 36.8% of the participants eat breakfast every day and 26.6% of the participants eat fruit every day, while 10% eat fruit only once or twice a week. Also only 10.8% of the participants drink milk every day. Only 20.8% of the participants said yes to the question of "Do you have any nutrition lessons?" Also there was a positive and significant correlation between diet and attitude of both female and male students. 22.8% of students were stayed at dormitory and 65.5% were stayed at home with their friends. No significant relationship was detected between the family income, (p=0.334), current place of resident (p=0.574). 90.3% of the students leave the meal and 6% of students leave the meal and 3% of student told that they didn’t have time. 70.1% of the students didn’t believe that they were healthily nourished. 20.6% of the students

340
prefer eating their meals at university refectory/dormitory-lodgings.

In this study was identified that the top five ranking foods consumed at high frequencies on daily basis included bread (93.4%), milk (44.9%), fresh vegetables (38%), cheese (35.4%) and egg (17.7%). While the top five foods indicated as never consumed by the students are fish (26.0%), lamb (25.4%) and beef (23.6%). The results from eating behaviour questions indicated that students in this study do not use a food guide to help them choose the food they eat. Consumption of fresh vegetables has been widely believed to promote good health; and protect human body from various diseases particularly those associated with deficiency of vitamins and minerals (Dietary Guidelines For Americans, 2014).

The frequency of consumption of milk and the frequency of consumption of nutrients in the fruit and vegetable group among students was under the necessary level. The most frequently consumed drinks were, successively water, tea and coffee. Results from eating practices showed that a high percentage of the students have unhealthy eating practices with less than or more than recommended dietary guidelines for most food groups therefore major changes in eating habits of this sample are required.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nutrition knowledge score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>n</td>
</tr>
<tr>
<td>Female</td>
<td>237</td>
</tr>
<tr>
<td>Male</td>
<td>381</td>
</tr>
<tr>
<td>BMI Classification</td>
<td></td>
</tr>
<tr>
<td>Thinness</td>
<td>86</td>
</tr>
<tr>
<td>Normal weight</td>
<td>169</td>
</tr>
<tr>
<td>Overweight</td>
<td>52</td>
</tr>
<tr>
<td>Obese</td>
<td>25</td>
</tr>
<tr>
<td>No reply</td>
<td>49</td>
</tr>
</tbody>
</table>

Findings regarding the association between gender and BMI and nutrition knowledge are reported in Table 4. Means and standard deviations of nutrition knowledge scores are shown to highlight gender and BMI classifications. Higher nutrition knowledge scores were significantly associated with being thinness or normal weight (p<0.002).

Regarding the percentage of correct answers according to different constructs, among the group of questions concerning food nutrients the best basic knowledge was found on the item regarding the definition of vitamins (57.8% correct answers) and the worst on the item about balanced diet (37.7% correct answers). Knowledge about food contents was found to be generally acceptable, with about half of the students responding correctly to all items with the exception of the one regarding pasta and bread content (52.7% correct answers).

In this research was found that there was a positive and significant correlation between the knowledge and attitude level of both genders. Also knowledge level of male subjects was higher than that of female subjects. There was also a positive and significant correlation between the nutrition attitude and practice (r=0.48; p<0.00). It was obtained knowledge level of food engineering, medicine and health science are highest and arts design and architecture sciences and vocational schools are lowest. Using the one-way ANOVA, there was a significant differences in the knowledge level between students of different educational programs (F=3.06; p<0.001). Findings showed that students’ knowledge of nutritional has a statistically significant influence on their nutritional behaviour (t=2.885, p=0.004<0.01).

**Principal Component Analysis (PCA)**

Principal Component Analysis (PCA) is recognised as being a powerful tool for pattern recognition, classification, modelling, and other aspects of data evaluation (Csomos et al., 2002; Škrbić, Onjia, 2002; Slavković et al., 2004). It eliminates the redundancy from the data, reducing their dimensionality by revealing several underlying components.

Initially, the aptness of the data for the PCA has been analysed with the KMO (Kaiser-Mayer-Olkin) test. The KMO value was 0.624, and the fact that the KMO value is higher than 0.50 shows that the variants are suitable for PCA and the number is sufficient. In addition to that, a global test has been made, according to the result; it has been shown that the samples drawn are at a level that can represent the population.

The principal components account for the total variance of the original variables (Table 5). The first principal component (PC1) accounts for the maximum of the total variance, the second (PC2) is uncorrelated with the first one and accounts for the maximum of the residual variance, and so on, until the total variance is accounted for. For a practical problem, it is sometimes possible to retain only a few components, accounting for a large percentage of the total variance.

Using the results of PCA, the four most important factors were identified, these being those that explained a high proportion of original variance and had Eigenvalue higher than one. These four factors combined to explain 68.25% of the total variance. The contribution of the variables to the main factors obtained in the PCA of components of nutritional habits and attitudes and variance explained are shown in (Table 5).

The data were analysed using factor analysis (Principal Components with Varimax Rotation). Factor Analysis reduced the 15 independent variables into four factor groups. Each factor group contains independent variables that are highly correlated with each other, but
no correlations exist among the factor groups. These factors can be defined as follows.

<table>
<thead>
<tr>
<th>Table 5 Principal components loadings for nutritional habits and attitudes</th>
<th>Factor Score</th>
<th>Percent of Total Variance Explained</th>
<th>Eigenvalue</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>F1</strong> (Dietary quality)</td>
<td>I'm carrying on a family tradition, my eating habits</td>
<td>0.828</td>
<td>27.21</td>
</tr>
<tr>
<td></td>
<td>I think I have a healthy diet</td>
<td>0.712</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I see my friends around me that the healthy diet</td>
<td>0.677</td>
<td></td>
</tr>
<tr>
<td></td>
<td>It is important that the taste of food is very nutritious</td>
<td>0.528</td>
<td></td>
</tr>
<tr>
<td></td>
<td>if you do enough exercise, you can eat whatever you like</td>
<td>0.701</td>
<td>18.64</td>
</tr>
<tr>
<td><strong>F2</strong> (Diet-related attitudes)</td>
<td>I have enough knowledge about balanced and healthy diet</td>
<td>0.627</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I eat three meals every day</td>
<td>0.596</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Food sold in the canteen affecting my eating habits</td>
<td>0.437</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sold in the canteen, toast, sandwiches, fast food more than I would prefer to eat school</td>
<td>0.431</td>
<td></td>
</tr>
<tr>
<td><strong>F3</strong> (Nutritional knowledge)</td>
<td>I think, I'm a balanced diet</td>
<td>-0.662</td>
<td>11.28</td>
</tr>
<tr>
<td></td>
<td>Generally, I prefer to eat the school cafeteria</td>
<td>-0.616</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Schools are required to sell only healthy foods</td>
<td>0.590</td>
<td></td>
</tr>
<tr>
<td></td>
<td>In terms of nutritional value of the meals in the cafeteria does not meet my needs</td>
<td>0.504</td>
<td></td>
</tr>
<tr>
<td><strong>F4</strong> (Healthy lifestyle)</td>
<td>I eat enough vegetables for my health</td>
<td>0.888</td>
<td>11.12</td>
</tr>
<tr>
<td></td>
<td>I do 30 minutes of physical activity at least five days of the week</td>
<td>0.870</td>
<td></td>
</tr>
</tbody>
</table>

The first factor summarizes four variables related to the *dietary quality* and explains 27.21% of the variance after varimax rotation.

The second factor contains five items that describe students' *diet-related attitudes* and explains 28.64% of the variance.

The third factor contains four statements and describes the *nutritional knowledge*. This factor explains 11.28% of the original variance.

The fourth factor summarizes two variables related to the healthy lifestyle and explains 11.12% of the variance after varimax rotation.

**Conclusion**

From this study it could be concluded that students should pay more attention to nutrition. Since university student will form the main body of families and professionals in every region and every society and they will represent the future parents (Bano et al., 2013). The time they spend at college is a golden period for learning and can promote nutrition knowledge, the attitude and practices of students. Therefore, an improvement in the learning environment related to nutrition, need to be emphasized on college campuses. Besides, media was the major source of information and not all students were aware of the health hazards of soft drinks and low intake of fruits and vegetables. The significant association between the students' study field and their nutritional knowledge magnifies the role of education. The students' attitude and practices needed improvement, emphasizing the need for further studies and a practical nutrition education programmes.

The main goal of nutrition plans is to obtain the appropriate and necessary nutrition to remain healthy, to be physically prepared and to lead a healthy life. For this reason to promote the health level of a society, the attitudes of its people must be taken into account. Given that one of the main goals of universities is to broaden the knowledge of the people in a society, the enhancement of the nutrition attitudes, knowledge and practices of its students is of high importance, as this will subsequently lead to a more food conscious society and more healthy people.

In order to remain healthy, physically active and enjoy a healthier lifestyle it is necessary to obtain good nutritional knowledge and implement it. Mitchell et al (1999) identified that people with normal weight have a more healthy diet and better points in terms of nutrition knowledge and attitudes compared the others. The knowledge, attitude and practice must be considered in people in order to promote society health. According to Elhassan et al. (2013) one of the main goals of universities is to broaden knowledge of people of the society, so enhancing the nutrition attitudes, knowledge and practice of students have high importance because this subsequently will lead to more food-conscious society and more healthy people especially young adults.
In this research, the concept of healthy nutrition has been planned and conducted in order to determine the manners and the behaviours of the students that receive education at Namik Kemal University, towards nutritional habits. A significant association was found between the study field of students and their nutritional knowledge magnifying the role of education. The factors that affect students’ nutrition selection, the evaluation of the concepts of healthy nutrition, personal opinions concerning nutrition levels, reasons of inclining towards healthy nutrition and research of the information obtained in line with the information sources about nutrition constitute the objective of this study. Some researchers have shown that nutrition knowledge was highly and positively related to the behavior toward nutrition (Mahe, 2000; Saegert, Young, 1983; Read et al., 1988).

The study indicated that, university students often miss meals, the most leaving out meal was lunch, the reason therefore was ‘don’t have enough time’ and they had an unhealthy nutritional pattern. Activities like conferences with participation on a voluntary basis appeared to be more effective than previous obligatory lectures on the eating habits of the students. Reflection on these findings has led to one possible conclusion that young population should be educated and encouraged to promote healthier diets and lifestyles.

References

AUTHORS LIST

Acar Burcu 24
Almeida Cátia F. F. 271
Alsina Ina 72; 144; 262
Aluc Mehmet 321
Andrade Sónia C. 51
Anskienė Lina 47
Antonenko Kristina 86
Arneborg Nils 223
Augspole Ingrida 150
Aydogdu Ayca 202
Barroca Maria João 327
Bartkevics Vadims 111
Bartkiene Elena 276
Basaran Burcin 307, 339
Basinskiene Loreta 276
Beitane Ilze 90; 234
Boca Sigita 172
Brunava Linda 72; 77
Budreckiene Ruta 311
Bundiniene Ona 155
Cakste Ilze 184; 248
Campanella Osvaldo H. 239
Ciekure Elina 61
Cinkmanis Ingmars 178; 184
Ciprovica Inga 117; 266
Coelho Jorge 280
Correia Paula M. R. 271; 280; 327
Dandena Ance 324
Degola Lilija 42
Delmukhametov Artem 315
Dimins Fredijs 150; 284
Dubova Laila 262
Dukalska Liia 99; 217
Duma Mara 253; 262
Eglite Aija 302
Erol Tuğçe 24
Feldmane Jana 266
Galoburda Ruta 172; 228; 248
Garipoğlu Hafize 318
Gedrovica Ilga 117
Gil Helena 280
Gonçalves Susete 280
Gramatina Ilze 37; 133
Guine Raquel P. F. 51; 271; 327
Güvendi Ömer 330
Hansen Åse Solvej 223
Ikauniece Daina 28; 32
Jaundzilake Liene 90
Jefimovs Aleksandrs 212
Jefremovs Arturs Niklavs 284
Jemeljanovs Aleksandrs 28; 32
Juhnevica-Radenkova Karina 127; 161
Juodeikiene Grazina 276
Jure Mara 81
Kalnina Solvita 133
Kampuse Solvita 212
Kampuss Kaspars 13
Karacabey Erkan 8
Karklina Daina 138; 188; 198
Kasparaviciute Ernesta 294
Kavaliauskaitė Danguolė 155
Kaymak-Ertekin Figen 243; 257
Kendirci Perihan 289
Khrustalev Evgeny 315
Kirse Asnate 138; 188
Klava Dace 123; 127
Klimienė Irena 47
Klapsaitė Dovile 276
Koç Banu 243
Koç Mehmet 257
Koscelkovskienė Irina 333
Kostova Maria 324
Kovalcsaks Aleksandrs 253
Krasnova Inta 127
Kreichbergs Viesturs 86
Kruma Zanda 150; 178; 192
Krumina-Zemture Gita 234
Küçüköner Erdoğan 8
Kuka Mara 248
Kuka Peteris 284
Kunkulberga Daiga 66; 86; 117; 133
Lepsa Liga 262
Lidums Ivo 188
Linde Raimonds 266
Linina Anda 66; 86
Lücke Friedrich-Karl 19
Lukosiuaitė Giedre 294
Lindhauer Meinolf G. 339
Melgaile Aija 61; 111
Mert Ilkem Demirkesen 239
Mierina Inese 81
Mikelsonė Velga 284
Mockeliūnas Raimundas 47
Muizniece-Brasava Sandra 99; 144; 217
Muratova Evgenia 94; 105
Murniece Irisa 206; 234
Nuobariene Lina 223
Onogur Tomris Altug 289
Özteş Semih 257
Özdestan Özgül 24
Ozolina Vija 117
Pavilonis Alvydas 47
Plančiūnienė Raimundas 47
Priechina Liga 198
Pugajeva Iveta 111
Pyanov Dmitry 315
Radenkovs Vitalijs 127; 161
Rakcejeva Tatjana 37; 66; 133; 144; 150; 167; 212
Rieknina-Dolge Rita 178
Roseiro Luísa C. P. 51
Ruse Karina 167
Ruža Antons 66
Ruzaike Aija 217
Ružauskas Modestas 47
Sabovics Martins 178; 228; 248
Sahin Serpil 202; 239
Salaseviciene Alvia 333
Saußerde Rudite 13
Schreiber Sonja 19
Seglina Dalija 161; 172
Semjonovs Paves 266
Senhofa Santa 123
Sério Susana A. 327
Shatalov Ivan 298
Shatalova Alexandrina 298
Shlekin Aleksandr 298
Silina Laima 37
Silins Indulis 55
Simoneliene Ausra 294
Skrabule Ilze 206
Skrivele Mara 161
Skrupskis Imants 172
Skudra Liga 37; 161
Smolikhina Polina 94; 105
Starkute Roma 155
Sterna Vita 28; 32; 72; 77; 117
Straumite Evita 123; 178; 228
Strauta Liene 144
Strazdina Vita 28; 32
Strele Maija 81
Struzeckiene Agne 311
Sumnu Gulum 202; 239
Šiugždinienė Rita 47
Tokareva Tatjana 302
Tomsone Lolita 178; 192; 206
Treciokienė Ernesta 294
Turgut Sebahattin Serhat 8
Unakitan Gokhan 307, 339
Ungure Eva 99
Vaiciulyte-Funk Lina 333
Vaivoke Aija 206
Valcina Olga 61
Vaškevičiūtė Lina 47
Vicupe Zaiga 72; 77
Virgailis Marius 46
Vojevodova Anna 81
Vucane Sanita 184
Vysniauskas Gediminas 294
Yalcin Buket 257
Yalcin Erkan 330
Yilmaz Emine 307, 339
Yilmaz Ismail 318; 321
Zalatorius Vytautas 155
Zeberga Sindija 81
Zeipina Solvita 262
Zuka Liga 324
Zumbure Laura 111
Zungur Ashi 257
Zute Sanita 72; 77
PUBLICATION ETHICS AND MALPRACTICE STATEMENT

Proceedings of the Baltic Conference on Food Science and Technology (FOODBALT) is peer-reviewed conference proceedings published by Latvia University of Agriculture (LLU). Proceedings of FOODBALT is committed to maintaining the highest ethical standards. Our ethic statements are based on COPE’s Code of Conduct and Best Practice Guidelines for Journal Editors. In order to provide our readers with conference proceedings of high quality, Proceedings of FOODBALT is guided by the following principles.

EDITORIAL BOARD

- The editors of Proceedings of FOODBALT are responsible for maintaining the integrity of the academic record, for having processes in place to assure the quality of the material they publish and for precluding business needs from compromising intellectual and ethical standards.
- The editors at any time evaluate manuscripts for their intellectual content without regard to the nature of the authors or the host institution including race, gender, sexual orientation, religious belief, ethnic origin, citizenship, or political philosophy of the authors.
- Editors ensure that each manuscript is initially evaluated by the editor for originality. After passing this test, the manuscript is forwarded to two reviewers for single-blind peer review, each of whom will make a recommendation to accept, reject, or modify the manuscript.
- The editors ensure that appropriate reviewers are selected for submissions (i.e. individuals who are able to judge the work and are free from disqualifying competing interests).
- The editors have systems to ensure that material submitted to their journal remains confidential while under review.

REVIEWERS

- The reviewers of Proceedings of FOODBALT assist the editors in taking the decision of publishing a submitted manuscript. By formulating suggestions to the authors, the reviewers can contribute to the improvement of submitted works.
- Any manuscripts received for review must be treated as confidential documents. They must not be shown to or discussed with others except as authorized by the editor.
- Reviews should be conducted objectively. Personal criticism of the author is inappropriate. Reviewers should express their views clearly with supporting arguments, so that authors can use them for improving the paper. Any selected referee who feels unqualified to review the research reported in a manuscript or knows that its prompt review will be impossible should notify the editor and excuse himself from the review process. Reviewers should not consider manuscripts in which they have conflicts of interest resulting from competitive, collaborative, or other relationships or connections with any of the authors, companies, or institutions connected to the papers.
- The reviewers should comment on the originality of submissions and should be alert to redundant publication and plagiarism.

AUTHORS

- The authors ensure that they have submitted original works, and if the authors have used the work and/or words of others that this has been appropriately cited or quoted.
- Authors should present an objective discussion of the significance of research work as well as sufficient detail and references to permit others to replicate the experiments. Fraudulent or knowingly inaccurate statements constitute unethical behaviour and are unacceptable.
- Submitting the same manuscript to more than one journal is considered as an unethical publishing behaviour and is unacceptable.
- Proper acknowledgment of the work of others must always be given. Authors should cite publications that have been influential in determining the nature of the reported work. Sources of financial support, if any, must be clearly disclosed.
- The corresponding author should ensure that there is a full consensus of all co-authors in approving the final version of the paper and its submission for publication.
- If the reviewers recommend extensive revisions, authors may be asked to revise their work and then to resubmit the article.
- Author must not use any fabricated research data in their work, which might lead to deceived outcomes.
- When an author discovers error or inaccuracy in his/her own published work, it is the author’s obligation to notify the journal editor and cooperate with him to retract or correct the paper.